10, 019, 243 (handwritten)

⑫ **EUROPEAN PATENT SPECIFICATION**

�54 **ANALYSIS METHOD AND APPARATUS FOR BIOLOGICAL SPECIMENS.**

㊶ References cited:
JP-A- 5 988 716   US-A- 3 297 879
US-A- 4 129 854   US-A- 4 174 178
US-A- 4 175 860   US-A- 4 207 554
US-A- 4 408 231   US-A- 4 513 438
US-A- 4 592 089

STEDMAN's MEDICAL DIRECTORY, 24th edition, Williams & Wilkins; pp. 778-779, 1329, 1331&NUM;

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Descripti n

The invention relates generally to the measurement of cell object features and parameters by image analysis, and is more particularly directed to quantitative measurement methods and apparatus for DNA analysis in small cell populations.

The present invention is directed to a quantitative testing apparatus and method which may be used for a wide range of diagnostic and prognostic evaluations of various cells, antigens, or other biological materials taken from the human body. However, for purposes of illustration and ease of understanding, the invention will be disclosed in conjunction with its preferred use, which is the quantitative measurement of cellular DNA for the purpose of cancer diagnosis and prognosis. More specifically, the present invention is directed to a method of interactive image analysis for analyzing and quantifying the DNA in specimen cells taken from a person.

The current state of the art in the pathology laboratory is to measure the DNA content of a cell by the visual observation of the pathologist who observes primarily the shape and texture of suspected cancer cells and who then classifies the cells into a normal category or into one of several abnormal cancer categories. However, such evaluations are very subjective and can not differentiate and quantify small changes in DNA within individual cells or in very small populations of abnormal cells. These small changes may represent an incipient stage of cancer or a change in cell structure due to treatment of the cancer by chemotherapy or radiation. Such small changes are, therefore, important in the diagnosis and prognosis of these diseases.

However, the advantage in diagnosis and/or prognosis of abnormal ploidy distributions that a pathologist viewing a specimen under a microscope has is the discerning expertise of a skilled person in classifying cells as normal or abnormal. There is an innate human ability to make relatively quick infinite gradations of classification, i.e., almost normal, slightly abnormal, etc. On the other hand, the classification and measurement of cell features and parameters by a pathologist on a cell-by-cell basis is extremely tedious and time consuming. Broad statistical analysis of such cell data taken by hand is relatively difficult because each record has to be entered and then processed. For different records, taken at different times, and under varying conditions broad statistical categorizations may be unreliable.

The alternative is automated cell analysis where the pathologist uses specialized equipment to perform the analysis. In automatic cell analysis, such as that accomplished by a flow cytometer, mass tests are performed in gross on a specimen cell population without a researcher being able to exclude or include certain data of the population. The specimen is measured "as is" without really knowing what cells are being measured and how many. Important single cell data or data from relatively small groups of cells are lost in the overall averaging of a specimen. Further, relatively large amounts of a specimen have to be used to provide any accuracy because of the averaging problem. This was considered necessary in the prior art to process large amounts of cell data relatively quickly so that the results will be fairly accurate. Again small changes in individual cells or small cell populations cannot be discerned.

Although there are commercially available general purpose flow cytometers, they are very expensive and can handle only liquid blood specimens or tissue disaggregations. These cytometers are incapable of working on standard tissue sections or using conventional microscope slides which are the preferred specimen forms of pathology laboratories. Additionally, a flow cytometer does not allow for the analysis of morphological features of cells such as texture, size and shape of cell nuclei and alterations in the nuclear-to-cytoplasmic ratios of cells.

Moreover, for such cell analysis, either automatic or manual, to be of real value there should be some way of verifying the results. The normal scientific method for accomplishing verification is to save the specimen so that another pathologist can compare his analysis to that of the first. However, for individual cells classified by manual means this indicates either photographs, drawings, or other imprecise mediums because it is extremely difficult to fix a tissue specimen for a long period of time. Further, even with those techniques where such specimens are fixed sufficiently for subsequent viewing, there remains the problem of finding the same cell or small population of cells from which an original evaluation was made and presenting the same conditions for viewing. With automated methods, the sample is consumed and verification can only occur by observing similar tissue from the same area.

Various computer-controlled cell analaysis systems are known. A computer-controlled cell classification system in which cell parameters can be measured by video signal is described in US-A-4129854. The apparatus sorts leucocytes into one of six categories and uses a chosen leucocyte as an internal reference to adjust the parameters for sorting leucocytes. However there is no teaching concerning calibration of the apparatus for variations in lighting and the apparatus cannot provide objective quantitation of any of the parameters of the cells.

Further computer-controlled imaging systems are described in US-A-4175860, US-A-4408231, US-A-4207554 and JP-59-88716.

US-A-4175860 is directed to the sorting of cells after Papanicolaou staining. However the Papanicolaou stain is a polychromatic stain and does not stain cellular constituents to allow objective quantification. The computing of a gray level histogram is described but calibration of the apparatus for stain and light variation is not mentioned.

In the apparatus of US-A-4408231 there is a circuit which maximises the useable video signal for a photosensitive scanning array for use in facsimile transmission and optical character recognition. The circuit varies light intensity to improve the sensitivity of the apparatus but cannot calibrate the machine to improve quantification of a parameter.

US-A-4207554 also describes apparatus for classifying blood cell types but again does not provide any means for quantification of the cellular materials.

Finally JP-59-88716 describes a method for automatically focusing on a glass slide by providing markers on the slide for automatic focusing. Again however there is no disclosure of calibration for staining variations or for light intensity.

In accordance with one aspect the invention provides a method of quantifying an amount of cellular material in an automatic image analyzing apparatus including the steps of:

providing a support means which has calibration material thereon, said calibration material having at least one parameter of a known value and having an optical density which varies in relation to staining concentrations;

providing specimen cell objects having an unknown value for at least one parameter on a support means;

staining the specimen cell objects and calibration material at the same time with an image enhancing material;

calibrating the automatic analyzing apparatus to adjust for variations in the amount of stains on the specimen cell objects in response to analysis of the staining of the calibration material;

forming a digitized image from the stained specimen cells on a support;

analyzing at least one parameter of said specimen cell objects using the digitized image thereof, said method being characterized by:

said calibration material having at least one parameter of a known value with said parameter correlating to an optical density which varies in relation to staining concentrations;

calibrating the automatic image analyzing apparatus for variations in light intensity by adjusting the apparatus in response to analysis of the intensity of light received by said image analyzing apparatus;

quantifying at least one parameter of said specimen cell objects using the digitized image thereof; and

reporting on the analyzed specimen cell objects based on the quantification and analysis.

In accordance with another aspect the invention provides an apparatus for analyzing cell objects in an automatic image analyzing apparatus including:

means for supporting calibration material and specimen cell objects, said specimen cell objects having an unknown value for at least one parameter;

means for calibrating the automatic analyzing apparatus to adjust for variations in the amount of staining of specimen cell objects in response to analysis of the staining of the calibration material, said specimen cell objects and calibration material having been stained at the same time with an image enhancing material;

means for forming a digitized image from the stained specimen cells objects on a support;

means for analyzing at least one parameter of said specimen cell objects using the digitized image thereof; and

means for reporting on the analyzed specimen cell objects bagged on the quantification and analysis, said apparatus characterized by:

said calibration material having at least one parameter of a known value with said parameter value correlating to an optical density which varies in relation to staining concentrations; and,

means for calibrating the automatic image analyzing apparatus for variations in light intensity by adjusting the apparatus in response to analysis of the intensity of light received by said image analyzing apparatus; and

means for quantifying at least one parameter of said specimen cell objects using the digitized image thereof.

The invention solves the problems of the prior art relating to the image analysis of various features and parameters of cell objects by providing a measurement method and apparatus which can acquire quantitative data concerning a plurality of individual cells very quickly by an interactive process with a

pathologist or an operator. The apparatus provides means for displaying an image of a group of cells from a field of a microscope slide. The image is further digitized and stored in a memory of the apparatus. From the digitized image a processor means identifies each possible cell object automatically by pattern recognition technique. An interactive program allows the operator to point to each object or cell in succession and make decisions for classification and measurements concerning each. For quantitative DNA analysis, the measurement is of the optical density of the cell object and the classification is by a pathologist as to whether the cell appears normal or cancerous. The decisions can include whether to accept or reject the particular cell for further processing. The cell object, if selected, can also be classified into one of several classifications for later statistical analysis. The apparatus further has means which provide for the classification and storing of more than one image.

In particular accurate quantitative measurements are facilitated by calibration for variation in stain concentration and light intensity.

One of the features the present apparatus provides is the enhancement of the image by providing a threshold value for the data prior to its display. The displayed image corresponds to a plurality of pixels which form the digitization of the image field. Below such threshold, a pixel in the display is shown as white or the absence of any information. A grey scale image above the threshold is displayed for the remaining pixels. This feature advantageously reduces background clutter and enhances the visual characteristics of the cell objects in the field. The threshold is variable and can be used to mask certain features while enhancing others. The full scale of grey resolution is used above the threshold for excellent contrast differentiation.

Another feature of the invention provides for the verification of the measured data. When each image or field is digitized and stored, a reference number is stored with the data. Preferably this reference number is the relative X, Y position of the image from a selected coordinate origin on a slide. The invention provides means for displaying the actual position of the area of a slide being viewed. Therefore, to verify a previously stored image, a slide is remounted with respect to a reference and positioned until its actual displayed position matches the stored reference position. Thus, the data and analysis of the study can be verified not only by the data images from a slide, but the actual slide image used in the analysis can be found readily.

When the apparatus is used for DNA analysis, tissue and cell specimens are applied to a slide which is then stained with a specific stain that combines proportionately with the DNA and essentially renders invisible the remainder of the cell so that the image analysis can measure the optical density of the DNA which is concentrated in the nucleus of the cell. The stain associates with the DNA to provide a detailed nuclear structure and pattern which may be visually observed and interpreted by the pathologist using the apparatus for classification. The amount of DNA in the malignant cells is substantially greater than that for normal cells because the malignant cells are usually dividing and replicating rapidly or the malignant cells have abnormal numbers of chromosomes or have defective chromosomes.

The preferred and illustrated apparatus of the present invention can not only detect minute alterations in the nucleus by providing a real and accurate measurement of the DNA mass in picograms but also can measure and quantify the amount of DNA and relate it to stored statistical analyses to aid in the diagnosis. More specifically, the invention allows an iterative analysis of specimen population cells and provides a histogram or other statistical display of the population distribution of the cells with respect to their DNA content and with respect to a standard DNA for normal cells so that subtle shifts in population distribution can be readily understood. To this end cell nuclei images are not only acquired and stored but the data therefrom can be integrated with other statistical data to provide multivariate analysis, discrimination of cells, histograms, and scattergrams of cells or cell populations.

The use of image analysis techniques and equipment for stained specimens by pathologists in a conventional pathology laboratory involves solving a number of problems which have been overcome by the present invention. For example, while there are a number of available staining techniques which can be used, such as an Azure A Feulgen staining technique described hereinafter, the staining of the DNA will vary substantially not only from slide to slide and from batch to batch by the same pathologist but also will vary substantially between different pathologists and different laboratories. Because the present image analysis apparatus measures grey level or optical density and because it is desired to provide a true actual measurement of DNA in picograms, it is important to overcome the problem of different staining factors for different specimens. Also, image analysis techniques which use adjustable microscopes and optical lighting provide different intensities of light when used by the pathologist. Trained researchers, in research laboratories may be equipped to adjust the optical intensity to the desired conditions for image analysis by image pattern techniques but this generally cannot be accomplished with the precision necessary in the usual pathology laboratory. Thus, there is a need to overcome the problem of this light intensity and, therefore, optical density variable.

Additionally, the present invention is directed to overcoming the problem of high costs heretofore associated with automated equipment used for image analysis; and to this end, the present invention provides a facile interactive system in which the pathologist performs a number of tasks and performs the preparation of cells and their selection by manipulation of the equipment. The pathologist also is provided with slides which are specially prepared and calibrated with reference cells to aid in the analysis of the specimen cells and to assist in overcoming the above-described staining problem.

The present invention has particularly been developed to locate cells for examination as to their morphology and to preserve their location for a later or corroborating analysis by a second pathologist when so desired. As will be explained, with respect to nuclei, measurements may be obtained as to their area in microns, total nuclear optical density or nuclear mass in picograms, average nuclear optical density, nuclear texture, and deviation of the nuclear shape from being a round nucleus.

Accordingly, a general object of the invention is to provide a new and improved method and apparatus for analyzing cells or other biological materials by using image analysis techniques.

Another object of the invention is to provide a new and improved method and apparatus for making a quantitative ploidy analysis of cells using image pattern recognition equipment.

A further object of the invention is to provide a new and improved slide or support for specimen cells having control cells or cell objects thereon which are used for calibrating the image analysis equipment.

These and other objects, features, and aspects of the invention will become apparent upon reading the following detailed description when taken in conjunction with the attached drawings wherein:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a pictorial representation of an image analysis system constructed in accordance with the invention;

FIG. 2 is a functional block diagram of the image analysis system illustrated in FIG. 1 which is adapted to perform an image analysis method in accordance with the invention;

FIG. 2A is a functional system diagram illustrating the major operations of the system control illustrated in FIG. 2;

FIG. 3 is an electrical block diagram of an interface electronics for the X, Y position circuitry illustrated in FIG. 2;

FIG. 4 are representative timing waveforms for the input to the interface electronics illustrated in FIG. 2;

FIGS. 5A, 5B, and 5C are perspective, top, and cross sectional views, respectively, of a slide particularly adapted for use in the image analysis system illustrated in FIG. 1 having separate areas for calibration cell objects and specimen cell objects;

FIGS. 6-9 are pictorial representations of histograms for different normal and abnormal cell populations;

FIG. 10 is a pictorial representation of the different screen images viewable on the instruction monitor illustrated in FIG. 1;

FIG. 11 is a pictorial representation of the main screen image which appears on the instruction monitor illustrated in FIG. 1;

FIG. 12 is a pictorial representation of the calibration image screen which appears on the instruction monitor illustrated in FIG. 1;

FIG. 13 is a pictorial representation of the analysis screen image which appears on the instruction monitor illustrated in FIG. 1;

FIG. 14 is a pictorial representation of the X, Y field coordinates image screen which appears on the instruction monitor illustrated in FIG. 1;

FIG. 15 is a pictorial representation of the option list for the main screen illustrated in FIG. 11;

FIG. 16 is a pictorial representation of the option list for the calibration screen illustrated in FIG. 12;

FIG. 17 is a pictorial representation of the option list for the analysis screen illustrated in FIG. 13;

FIG. 18 is a pictorial representation of the measure operations for the calibration screen illustrated in FIG. 12;

FIG. 19 is a pictorial representation of the analysis operations for the analysis screen illustrated in FIG. 13;

FIG. 20 is a pictorial representation in time sequence of the analysis operation for a field of cell objects as seen on the image display illustrated in FIG. 1;

FIG. 21 is a pictorial diagram of a slide used for image analysis which has been divided into a plurality of selectable image fields by the image analysis system illustrated in FIG. 2;

FIG. 22 is a pictorial diagram of a histogram used for light source calibration in the image analysis system illustrated in FIG. 2;

FIG. 23 is a flow chart diagram of the program which reads the X, Y coordinate position of the platform of the microscope illustrated in FIG. 1;

FIG. 24 is a software flow chart of the program whose function is to process the analysis and measurement operations for the analysis and measurement screens illustrated in FIGS. 12 and 13, respectively, and

FIGS. 24A, 24B and 24C, illustrate a field array of several cell object tables, a cell object array and a cell object table respectively as utilised by the program.

The apparatus illustrated in FIGS. 1 and 2 can be used to develop histograms, and other statistical data, of cell populations for the diagnosis and prognosis of malignancies and other diseases. To illustrate the utility of such statistical analysis reference is directed to FIGS. 6-9.

Referring now to FIG. 6 there is shown a normal ploidy histogram having cell number versus mass distribution for healthy, non-dividing cells. The number of cells is provided on the ordinate axis and their nuclear mass on the abscissa. If the cell population shown in the figure is not dividing, the DNA content should be peaked around a normal peak G0/G1 which is the diploid amount, 7.18 picograms/cell. This relative mass of DNA is labelled as 1.0 to normalize the abscissa of the histogram. FIG. 7 also shows a normal cell population which is dividing, where there is a significant G0/G1 peak at 1.0 and a second peak G2 at 2.0. The peak at 2.0 is normal because some of the cells are in division and have double the normal diploid amount of DNA. The saddle S between the two peaks is relatively low and does not indicate any malignancy.

Comparing the histogram in FIG. 8 with the first two, it is seen that this cell population is skewed from normal having a higher first peak around 1.5 and a second peak around 3.0. Further, the saddle S is more pronounced and can be rough in cell count. This histogram may show a malignancy because of the abnormally high DNA content of many of the cells. This high DNA content is likely indicative of the increased ploidy amount of malignant cells which are rapidly dividing. Likewise, in FIG. 9 it is shown that the G0/G1 peak occurs at 1.0 with a normal diploid amount of DNA but has a relatively large trailing saddle from 1.0 to 2.8. A normal G2 second peak is not noted and is indicative of an abnormal cell population. The shape of the histogram is likely due to abnormal DNA amounts in cells and clones of cells indicative of malignancy. Therefore, from the shapes and changes in cell distribution histograms, diagnostic and prognostic information can be obtained.

In the implementation shown, the system is a computerized image analysis system designed to measure a number of cell object features and parameters from their image on a typical glass slide. The instrument includes a sophisticated digital image processing system controlled by software to perform quantitative analysis on individual cells for nuclear DNA content by Feulgen staining as well as measurement of other nuclear features. The imaging system couples the ability of a pathologist to identify cells to be studied with the capability of computer enhanced, high resolution digital video image processing to quantify optical and stain density accurately.

In general, a pathologist first prepares a touch preparation or a needle aspirate of fresh tissue. Alternatively, embedded samples can be visually inspected for areas of interest and then deparaffinized and disaggregated by mincing in the presence of pepsin to produce a single cell suspension that is then placed on a microscope slide. After fixation and staining with Azure A Feulgen stain, the preparation is ready for analysis.

The operator has the option of classifying the cells morphologically into any one of six categories or rejecting inappropriate cells or debris. The cell data are processed by a system control and the cellular elements are characterized by a quantitative DNA analysis for each cell class. The information when compared with either a standard cell calibration or published data allows a pathologist to accurately quantify and classify abnormalities that might ordinarily be described only verbally from the image a person sees.

The addition of quantitative data enables pathologists to perform their work in a more standardized and reproducible manner. The system is of value in classifying lesions that may be malignant and in providing prognostic information for known malignancies based on DNA content. The image identification system is more advantageous than common flow cytometry methods of evaluating DNA content. Flow cytometry permits an operator to classify neoplastic cells based only on cell markers. The pathologist, however, never sees the cells that the instrument has examined. In addition, the cell preparation must be used in a short time frame and is consumed in the course of the study. Although a permanent section of a tumor under study may be examined at the same time, there is no guarantee that the same cells are examined in both areas. Also the quantity of tumor available may not be large enough to permit a flow cytometric examination.

In the invention, the quantitative DNA analysis is performed rapidly for the measurement of DNA and ploidy distribution pattern in a cell population under study. The pathologist advantageously selects the cells

which are to be used in the population measurements. The measurement of DNA content is useful and believed to be relevant in diagnosing and determining prognosis for a variety of tumors that involve the breast, colorectum, and prostate. The system takes advantage of the pathologists skill to identify visually abnormal cells and then uses a computer aided imaging analysis to analyze quantitatively those particular cells for the parameters desired. Such instrument extends and augments the recognition and diagnostic skills of the pathologist.

As shown more specifically in the drawings for purposes of illustration, the invention is embodied in a method and apparatus for automatically analyzing "cell objects" which term is used herein to be generic to cells, such as blood cells or cells taken from tumors or the like, which are to be analyzed for the DNA content. The term is also meant to encompass non-biological objects, such as conventional plastic or glass spheres used in biological studies, painted cell images on a slide, or antigens or monoclonal antibodies on cells. The system further finds use where a monoclonal antibody is conjugated with a stain, wherein the stain may be a fluorescent material which is excited at one wavelength and which can then be analyzed at another wavelength where fluorescence occurs. By way of example, the present invention is useful for ploidy analysis, red blood cell analysis, pap smear cell analysis, monoclonal antibody analysis, and the analysis of other infectious diseases which can be diagnosed by DNA probes for viruses. The imaging and analysis system is, therefore, advantageously used in a number of studies where one of the optical characteristics, such as optical density, of a cell object may be used to determine some parameter or feature of that object which is diagnostic or prognostic for a particular condition.

As shown in FIGS. 1 and 2 of the drawings, the invention is embodied as an apparatus 11 which functionally operates as a digital image processing system 13 (FIG. 2). The apparatus 11 comprises a high resolution microscope 15 with which an operator can view specimens on a support, in the preferred embodiment a glass slide 14. The microscope 15 has means 70 for focusing its optics on the slide and a platform 51 movable incrementally by means 110 and 17 in X and Y directions for viewing various areas thereof. The specimens or material on the slide 14 are further viewable by the imaging system 13 which is controlled by a system control 22 in the form of a digital processor such as a personal computer. An operator can communicate with the system control 22 via a keyboard 36 and interacts with the apparatus 11 by viewing two displays. A first display, image display 37, is a RGB monitor which displays through the system control 22 the same image as seen through the microscope 15. A second display, instruction monitor 62, is another RGB monitor and is used to provide the operator with interactive prompts, messages, information, and instructions from the program controlling the system control 22. A printer 38 is provided to produce a reliable hard copy output of data produced by the apparatus 11.

The functional schematic of the apparatus 11 is illustrated in FIG. 2 as image processing system 13. The image processing system 13 is used to analyze a plurality of cell objects on the support or glass slide 14 of the microscope 15. Suitable high resolution microscope optics 16 receive light from a variable intensity source 17a through the slide 14. The optics 16 form an optical image of each of the cell objects on the slide 14 and transmit them to a image splitter 25 which can take the form of a prism.

On one side of the splitter 25, a television camera 18, or other detector, converts the optical images point by point into a scanned electronic signal representing the optical intensity of each point in the image. The output of the camera 18 which is a standard NTSC analog video signal is applied to an analog to digital converter of an image processing interface 21. The image processing interface 21 converts the image signal from the television camera 18 to a digitized signal which is received and stored by the system control 22. Because of the continuous scanning, a real time image of the area the optics 16 are focused on is provided by the image display 37. In general, the image is a 512 X 512 array of pixels each having a measured light intensity.

On the other side of the image splitter 25 is located the viewing optics 23 of the microscope 15. This parafocal arrangement allows the same image in the viewing optics 23 to be displayed on the image display 37. This feature allows the positioning of the platform 51 by the manual X, Y adjustment means 110 and 17 until the operator views a field of interest on the slide 14. At that time, a computer enhanced digitized image of the selected field is displayed on the image display 37 for further analysis.

Both of the displays 37 and 62 are controlled by the system control 22 through standard video interface circuitry 39 and 610, respectively. Similarly, the keyboard 36 and printer 38 communicate with the system control 22 through conventional interface circuitry 35 and 41, respectively. In addition, the system control 22 controls a random access memory 73 and bulk storage in the form of floppy and hard disk drives 75 through a memory control 71.

All of the interface circuits 21, 35, 39, 41, 610, 71, and 106 can be selectively embodied on printed circuit boards which are mounted in the backplane or card connector of a conventional personal computer forming the system control 22. Preferably, the personal computer can be one manufactured by the IBM

Corporation having a model designation AT. Such system control can be run under a disk operating system such as PC DOS version 3.1 or later. The system software for the image analysis is called as an application program from the disk drive 75, and could, for example, be supplied on a floppy disk 77. The system software is read from disk 77 and loaded into RAM 73. After loading program control is transferr d to the analysis software to regulate the various hardware elements previously set forth in a known manner.

5   The analysis software can be for DNA analysis as hereinafter described or could include other software for various purposes. For example, the analysis of the shape and size as well as hemoglobin content of the cells when examining red blood cells may be accomplished in accordance with the pattern recognition technique and analysis method disclosed in U.S. Patent Nos. 4,097,845 and 4,199,748 issued to Bacus which are hereby incorporated by reference as if fully reproduced herein.

10   FIG. 2A illustrates functional operation of the system software where control logic for the instrument hardware, the image display, and the instruction display are used to perform the main system functions. The main system functions are patient labelling, light level calibration, control cell calibration, cell data acquisition, cell classification, cell analysis, and report generation.

15   The interface circuitry, except the interface electronics 106, can be standard control circuits for the various functions illustrated. The interface electronic 106 is a specialized circuit which is more fully illustrated in FIGS. 3 and 4. The X, Y position sensors allow the field under analysis to be given a position.

Herein the X and Y locations of each field are easily determined for any given location by a novel method and apparatus which includes, as best seen in FIGURE 2, an X direction sensing strip 100 which 20   may be fastened to the underside of the microscope stage 51 for movement with the stage 51 past a sensing pad 102, which is secured to a stationary part of the microscope. The sensor provides an analog output to interface electronics 106 which provides the X coordinate in digital numbers to the system control 22 for storing in memory and for displaying on the monitor screen 62. Likewise, a similar strip 114 is fastened to the stage 51 for movement in the Y direction with the stage past a sensing pad 112 which is 25   secured to a stationary part of the microscope so that the pad may provide an analog signal to the interface electronics 106 which supplies digital signals to system control 22 for storage of the Y coordinate and for showing the Y coordinate on the video monitor adjacent the X coordinate. Manifestly, the system can be reversed with the sense heads fastened to the stage for movement therewith and with the sensor strips 100 and 110 being mounted stationary to provide analog signals as the heads move thereacross.

30   The circuitry for interfacing with the position sensors is more fully detailed in FIGS. 3 and 4. The position sensors are magnetic sensors having the pair of sensor strips 100 for the X coordinate and the pair of sensor strips 114 for the Y coordinate. The sensor strips 100 and 114 are affixed orthogonal to each other such that these strips form a reference axes for the coordinate system. The moveable sensor pads 102 and 112 which are affixed to the moveable base of the microscope 15 travel along the strips 100 and 35   114 sensing the relative movement between the members depending upon the relative position of the moveable pad with respect to the fixed strip. The sensors are connected to a measuring chip 180 which includes circuitry for measuring the relative position between the strips and for generating a digital numbers based on that sensed parameter. The X sensing strips 100 are connected to the sense XA and sense XB inputs of the measuring circuit 180 and similarly the Y sensing strips 114 are connected to the sense YA 40   and sense YB inputs of the circuit. The circuit 180 is an internally timed position to digital number generator which outputs a 3 byte serial digital number depending upon the relative position of the moveable pad with respect to the fixed strip from outputs OXA and OYA. This 3 byte number is serial and is accompanied by a clock signals XCLK or YCLK for timing purposes.

Each of these bursts of 24 bits are at a frequency of approximately 24 KHz. and occur every 100 45   milliseconds. The measurement circuit 180, therefore, produces a 3 byte number for the X position every 100 milliseconds and a 3 byte number representative of the Y position every 100 milliseconds. The outputs OXA and OXB are connected respectively to the serial input IN and clock input CLK of a shift register 20. Similarly the output OYA and output OYB of the circuit 180 are connected respectively to the serial input IN and clock input CLK of shift register 220. The signal XCLK will clock or time the 24 bits of the position 50   number into the shift register 20 every 100 milliseconds. This number is then stored in the shift register 20 until it is to be used by the main control program. The Y position of 24 bits is serially presented to the input of the shift register 220 and clocked in to the device by the signal YCLK every 100 milliseconds. The shift registers 20 and 220 act to hold these position numbers between communication bursts from the measurement circuit 180.

55   The shift registers 20 and 220 are connected to the system control 22 by the address lines A0-A15 of its address bus and by its data lines D0-D7 of the data bus. The 8 bit data bus is connected in parallel to the byte outputs A, B, and C of the shift register 20 and the byte outputs A, B, and C of the shift register 220. Each output represents one of the bytes of a 24 bit position word for either the X or Y position. The

outputs are enabled by the input lines A, B, and C connected to the outputs Q0-Q2 of a decoder 24. The address bus lines A0-A15 are connected to the input lines of the decoder 24.

The decoder 24 translates a specific address assigned for each byte of the position words and enables that byte from the respective shift register so that it can be read in on the data bus 28. For example, to read the X position, the system control 22 would output the address which is used by the decoder 24 to enable the A output of the shift register 20 and then read that byte in through the data bus 28. Thereafter, the system control 22 would output the address which would enable the B output of shift register 20, and then read that byte from the data bus 28. Subsequently, the system control 22 will output the address to enable the C output of shift register 20 and read that byte. The operation is identical for reading the Y position word from the shift register 220.

The reading and loading of the shift registers 20 and 220 is entirely asynchronous. Because the positions of the X and Y coordinate are updated every 100 milliseconds, a relatively simple transfer scheme like this can be implemented. If the shift registers 20 and 220 are shifting when the position words are being read by the computer, then the software will make suitable comparisons to determine whether the input number read is a correct number or the shift register was inputting another number when it was read.

A flow chart of the subroutine which reads the shift registers 20 and 220 is more fully illustrated in FIG. 23. The subroutine may be called periodically to determine the actual X, Y position of platform 51. The program begins by reading and storing the X position twice in blocks A225-A231. A comparison is made to see if the two are equal (A = B) and, if not, loops back to the start. A third read and store in blocks A235 and A237 precedes a comparison of A and C in block A239. A negative branch begins the process once again while a positive branch starts the reading of the Y position in the same manner. The technique requires three reads to match before the number is accepted as the correct position, thereby eliminating the possibility that the table was moved or the shift registers shifted between reads.

Because the apparatus 11 may be used in various offices such as pathology offices having persons of varying degrees of skill and knowledge about image analysis, the microscope light source 17a may be variously adjusted by different operators such that the background may have a different light intensity not only from machine to machine but also at different times depending on the age and nature of the lamp doing the illumination. When the cell objects are DNA nucleus, the stained nuclei appear darker and have high darker gray levels than the cells which have fewer or no DNA content. The particular light intensity level is desired to be known in an accurate and real manner; and hence, it is important that there be a calibration of the light intensity to eliminate errors which might be introduced if differences in light intensity levels are not accounted for.

A further problem with widespread usage of equipment of the foregoing kind is the staining factor by which is meant that the user may be applying either a heavy amount or a light amount of the Azure A stain. This will result in a variation of the gray level being viewed through microscope 15 and by the camera 18 which is then analyzed as to the particular DNA content. Thus, there is a need that the apparatus 11 be calibrated to eliminate differences because of the staining factor so as to provide a true indication of the actual amount of hemoglobin, DNA, or antigens or monoclonal antibodies on cells, etc. being analyzed.

In accordance with the present invention, calibration material 40 (FIG. 5A) is provided on the slide 14 which, when viewed by the operator under a calibration step of the system software allows the operator to adjust and to calibrate the apparatus prior to the measuring and analyzing of specimen cell objects 12 on the slide 14.

In the illustrated embodiment of the invention there are provided two different calibrating materials on the slide 14 with the first calibrating material being the control cell objects 40 which are stained simultaneously with the staining of the specimen cell objects 12. The simultaneous staining permits the analysis of the control cell objects to be compared to a predetermined stored reference light intensity, gray level, or optical density which the control cell objects 40 have after staining. If the cell objects are stained either too lightly or too heavily, the amount of understaining or overstaining can be quantitatively analyzed and adjusted for as will be described hereinafter.

In the preferred embodiment of the invention, the calibration material also includes an optical density reference material 45 which is usually a printed mark on the slide which has a predetermined known optical density, which can be used as a reference to calibrate the instrument. As will be explained in greater detail hereinafter instructions are provided to the operator from the system software to the operator. Following these instructions, the operator manually adjusts the background light intensity of source 17a until the desired intensity is obtained for the reference material 45. As will be explained hereinafter, this step provides the system control 22 with a calibration to read the proper optical density of the objects on slide 14.

As a safeguard to the integrity of the system, it may be desired to provide an integrity check or identification step for the slide 14 by analyzing a predetermined and prefixed optical pattern on the slide which is read and measured as to gray levels and physical dimensions before the analyzing may be begun. Herein, the optical integrity pattern may be in the form of initials CAS located above the control cell objects as seen in FIGS. 5A-5C. Manifestly, the integrity check may be the cross 52 or any other shaped material on the slide 14.

The light calibrating material 45 which is in the form of a cross 52 shown in FIG. 5B may also take a large number of different shapes and forms, and in fact, may be merely the border 54 for the control cell objects or may be a logo CAS or other identifying mark which has been applied to the slide to provide a predetermined optical density when the light source for the microscope 15 is adjusted to the desired intensity.

The present invention is also useful for later analysis of the specimen cells 12 on the slide 14; and to aid in the recall of cell images stored in memory or to allow the operator or another person to return to a given cell for a second review thereof at a later time. To this end, after the slide 14 has been secured on the microscope stage 51 (FIG. 1) in a particular position, a certain location or landmark on the slide, such as the center of the cross 52, is defined as a zero-zero X-Y reference point. This reference point is used to set up a translation table for the position readings from the X, Y sensors. A pair of location registers of the system control 22 for the X and Y distances are zeroed at this point so that subsequently all cell locations may have a specific X and Y coordinate address from the reference point. Another easy landmark to find with the position adjustment means of the microscope stage 51 is a corner such as the righthand lower corner 53 of the box border 54 within which are located the reference cell objects 40. The box border 54 is printed on the slide and it also may be used for the optical density calibration rather than the special cross 52. By suitable logic, any point on the slide and microscope stage at which the classification operation begins may be taken as the zero X and Y location with the location registers. The X and Y coordinates are initially zeroed at this location and then provide a readout for each image field location from this zero location.

The specimen slide 14 may be of any size or shape but because of the familiarity of laboratory technicians and pathologists with glass slides used with microscopes, it is preferred that the support 14 be an actual microscope slide of glass which typically measures 7.62 x 2.54 cms (3 inch by 1 inch). The illustrated slide 14 shown in FIGURE 5A has a preprinted border 54 within which are located reference or control cell objects 40. The slide also has an area 61 for specimen cell objects.

The control cell objects are, in this illustrated embodiment of the invention, lymphoblastoid cells of a known size and shape, and DNA content. The lymphoblastoid cells may be mostly of the type having normal DNA content, although some cells may have double or other ratios to the normal DNA content which is typical of cancer cells. The control cell objects may be other types of cells having dark centers or nuclei which stain well, such as chicken blood cells or trout cells. On the other hand, the cell objects 40 may be artifacts printed on the slide to have a cell shape. Furthermore, as above explained, the cell objects 40 may be conventional plastic beads of a predetermined size which will react with a particular fluorescent stain or enzyme stain when treated simultaneously with specimen cell objects such as monoclonal antibodies used in the specimen area 61 of the slide. The reference cell objects will vary from test to test and the present invention is not limited to any particular test or cell objects.

A pathologist will take a previously prepared slide such as shown in FIGURE 5A having premounted thereon the control cell objects 40 and add thereto the specimen cell objects 12 which are, in this instance, cells from a slice of tissue (such a tumor tissue), or a a needle aspirate of tumor tissue or monolayer of blood cells or other cells, at the area 61 on the slide. The pathologist will then stain or otherwise treat simultaneously the control cell objects and the specimen cell objects for image enhancement. The preferred slides are provided with a kit which have bottles of reagent therein specific to the control cell objects. For DNA analysis, the kit contains bottles of Feulgen Azure A reagent solution and bottles of rinse reagent solution to specifically and quantitatively stain nuclear DNA.

To prepare a slide, the following process is used. The slide 14 has normal control cells on one area and a space for the specimen cells on another area. The slide 14 is fixed within 10% formalin for ten minutes and then put aside while routine paraffin embedded sections are prepared for the area 61. If a malignant or questionable tissue is present on the permanent section, the slide 14 will be processed to analyze the section.

Processing consists of first treating the slide for 65 minutes in 5N hydrochloride to hydrolyze the nuclear DNA. The slide is then transferred to a container of Azure A stain for a two hour staining period. Afterward, the slide is washed in rinse solution, dehydrated with ethanol, cleared with Zylene, cover slipped, and mounted. At this point, the slide is permanently fixed and ready for analysis at any time.

The system software for DNA analysis can now determine the density of the cellular DNA by obtaining the optical density of the specimen cells via the instrument 11. In general, the mass of a stained cell object can be obtained from its optical density by utilizing Beer-Lambert Law which is well known in the art of microspectrophotometry. The equation states:

$$M = \frac{\alpha \sum OD}{E\lambda}$$

where

    M   = mass of the object in picograms

    α = spot size in μm^2

    $E\lambda$ = extinction coefficient of the stain at wavelength λ in $\mu m^2/pg$.

    OD   = optical density of each spot (dimensionless)

The instrument uses this law to find the mass distribution of a number of cells or cell objects which can then be analyzed according to a statistical basis, nistogram, or other analytical format as will be discussed hereinafter. The spot size α is determined by the number of pixels which are measured by the camera 18. The optical density for each pixel is calibrated by adjusting the light level, focus, and reading a reference optical density from the calibration area on the slide. This calibration allows the conversion of the measured light levels for each pixel into an optical density a dimensionless quantity.

A calibration for the extinction coefficient is accomplished by measuring the optical density for a plurality of the control cells to determine a peak for the distribution in relative mass units. Because the peak DNA content is known for the control cell distribution, the cells in the measurement field can be measured using the relative OD units and then converted directly into picograms by using the control cell calibration. For example, if the control cells are known to contain 5.96 pg of DNA (trout cells) and a group of calibration cells show a peak distribution of 11,000 relative OD units then a normal group of human cells (with a known DNA content of 7.18 pg.) would exhibit a peak in their distribution at approximately 13,250 relative OD units. Further, any other relative OD unit measurement can be converted directly into picograms by determining and using the extinction coefficient found from the group of calibration cells.

The system software for DNA analysis is a menu driven program that uses interactive information screens on monitor 62 to assist the operator in making the described measurements on several cells or cell subpopulations. In FIG. 10 there is illustrated the visual screen structure of the program which appears on monitor 62. A main screen 150 provides information as to the calibration and analysis status of the apparatus and provides an option list for calling three other primary information screens. A pictorial example of the main screen is shown in FIG. 11. The three primary information screens correspond to the three operational functions of the program where the label screen 156 corresponds to the label function, a calibration screen 152 corresponds to the calibration function (optical density and staining), and an analysis screen 154 corresponds to the analysis function. Each screen 152, 154, and 156 provides an option list or menu wherein one of the options is to exit to the main screen 150.

In addition, the operator can enter the calibration screen 152 from the analysis screen 154, or vice versa. Two other screens are available as options from the analysis screen 154 and are used to adjust the boundary of the viewing field with the adjust boundary screen 158 and to display the X, Y coordinates of the fields that have been measured with the X, Y fields screen 160. A pictorial representation of the calibration screen is illustrated in FIG. 12, while pictorial representations of the analysis screen and the X, Y field coordinates screen are illustrated in FIG. 13 and FIG. 14, respectively.

When the unit is in operation the pathologist has a number of options or functions from a menu on each screen from which he can choose to acquire data and process that data. In general, the program is menu driven from the main screen illustrated in FIG. 11 which provides a main menu of options as shown in FIG. 15. The main menu A10 consists of five main screen functions including a label function A12, a calibrate function A14, an analyze function A16, a help function A18, and an exit function A20.

The label function allows a user to enter information regarding patient identification, accession number, and DNA conversion number. The DNA conversion number is the number that the first and second peak masses are divided by to get the first and second peak indexes (FIG. 11). Initially, the number is set to a standard 7.18 picograms/cell for normal human cells. However, the apparatus may be used to measure non-human cells and the index may be changed to that desired. The DNA index number must be greater than or equal to 1.0 and less than or equal to 99.99. If the conversion number is not within that range, the user is not allowed to select the analyze option in either the main or the calibration screens.

The three lines of information entered during the label function will appear on every screen except the X, Y field coordinate screen. The label operation is exited by pressing either the enter or escape key. Pressing the enter key will save any changes that were made to the three lines of information. Pressing the escape key will ignore any changes that were made to the three lines. The information stored during the label function will not be saved when the program is exited.

Selecting the calibrate function will cause a change of the display on monitor 62 from the main screen to the calibration screen illustrated in FIG. 12. The calibration screen whose options are shown in FIG. 16 are those necessary to perform calibration of the instrument for optical density and for staining factor on the control cells. A calibration of the apparatus 11 is to be performed every time a new slide is selected to normalize the light level and staining factor.

Selecting the analyze function will cause a change of the display from the main screen on monitor 62 to the analysis screen as illustrated in FIG. 13. The analysis screen contains the menu for the functions that are necessary to perform data acquisition and DNA measurements on the specimen cells. These functions are more fully set forth in FIG. 17. Three criteria must be met in order to select the analyze function. First, the set light function in the calibration screen must have been successfully performed at least once. The set light function is successful when the current image is blank and the light level is between 129 and 131. Secondly, the calibration control cell count must be between 50 and 512. Finally, the DNA conversion number must be greater than or equal to 1.0 and less than or equal to 99.99.

The exit function allows the user to terminate the operation of the program from the main screen. Pressing the escape key is the same as selecting the exit function, When the exit operation is specified, either by selecting exit or pressing escape, the user will be asked to confirm his command to exit. To accept the confirmation, the user selects the yes key. To reject the confirmation, he selects the no key or presses the escape key.

The options for the calibration menu are illustrated in FIG. 16. The calibration menu choices include a set light function A24, a set X, Y function A26, a focus function A32, a measure function A36, an X, Y function A38, an analyze function A28, a clear function A30, a help function A34, and an exit or return to the main screen function A40.

The set light function A24 calculates the background light level for the current image. The light level can be adjusted until it is within a standardized range. The set light function should be successfully performed at least once before selecting the analyze, focus, and measure functions. For the most accurate results, the light level should be exactly equal to 130. The light level value is displayed on the calibration screen by the words "light level." If the light level is successfully set, image noise subtraction will occur in the measurement program.

The light source calibration achieves a number of results including assistance in focusing the microscope by providing an update of a gray scale histogram which may be in the form generally shown in FIG. 22 entitled "Distribution of Light Intensity". The illustrated histogram shows a comparison of incident light I_o and transmitted light I_t with the transmitted light having grey level value and the incident light having a grey level value. The operator has moved the platform 51 to view the light calibrating material 45 on the monitor 37. The operator views the light calibration material 45 and the system calculates a histogram of that pattern by inputting light from the picture elements of that field. The operator then adjusts the intensity level of the source 17a to change the light level reading on the screen. The operator does this by alternatively selecting the function and adjusting the source 17a until the correct reading appears on the screen. When the light source has been adjusted to provide the left and right peaks I_t and I_o for the transmitted and incidental light at the desired grey levels, 130 as the background light intensity, the system is calibrated. The system software of the apparatus 11 uses the I_o and I_t values to set up an internal calibration table for optical density, such that light intensity sensed for each picture element is referred to this table and known to have a particular optical density in the image scene being analyzed.

As is known with digital imaging apparatus the actual optical density for a dark object is known using white (I_o) as a reference. By calibrating the apparatus for optical density, the incoming image data may be converted by the lookup tables in the image processing interface 21 so that output optical density can be linearly added to proportionately reflect directly, in this instance, the amounts of DNA in the specimen objects.

The set X, Y of function A26 provides the setting of the origin for the slide X, Y coordinate system. This function sets the current image or field location as the origin by zeroing a pair of location registers in the software. Generally, the microscope platform 51 is moved until a easily recognized landmark is visible, such as cross 52. This landmark is then used to rezero the coordinate system to provide a means of relocating previously measured fields. The set X, Y function is used every time a new slide is selected. If the set X, Y function has not been executed, then the X, Y functions of the calibration and analysis screens and the

functions in the X, Y field coordinates screen will not work properly. The set X, Y function can only be used when the calibration control cell count is equal to zero. If the microscope platform 51 is being moved when the set X, Y operation is in execution, then the coordinate origin will be in error. The program provides a message on the screen to notify the operator when the set X, Y operation is successful. In response to the function not being successful, the operator merely reselects set X, Y from the menu and attempts the function again.

The measure function A36 is used to perform the control cell or control object calibration for normalizing the staining factor. When the measure function is selected, the camera image acquisition will stop and the cursor 170 on the calibration screen will move to the words "measure operations" in FIG. 12. When the cursor 170 is at this location, the user can specify measure operations by activating the numeric lock. An identifier such as a magenta colored box will be placed around an identified cell object. By using a number of key operations illustrated in FIG. 18, the operator can perform an interactive selection and rejection process which will be more fully explained hereinafter.

During control cell calibration, the operator moves the microscope stage by turning the conventional X and Y knobs 110 and 17 (FIG. 1) to shift the control cell objects 40 into view on the monitoring screen 37. When an individual cell object 40 is within a box or indentifier border, the operator presses a key on the keyboard 36 to enter measurement of the summed optical density for that control cell object. After a suitable number of control cell objects have been analyzed, the operator will be provided with a histogram such as shown in FIG. 12 on the video monitor 62 which shows the operator the control cell object ploidy distribution as having a relative quantity of DNA. Internally within the system control 22, the summed relative optical density values actually measured for the control cell objects is compared to a predetermined standard or reference amount of DNA which the control cells are known to have. In the present example, if the control cells contain 5.96 picograms of DNA per cell, then an optical density measurement of approximately 8700 relative OD units corresponds to that mass. The actual summed optical density found by the operator is divided into the stored reference DNA value to provide a factor by which to adjust the extinction coefficient for deviations in the stain from a perfect staining.

The X, Y function A38 when selected displays on the calibration screen, the X, Y coordinates of the current image or field, on monitor 37. The coordinates will be continuously displayed until the user presses a key (except CTRL, ALT, or SHFT). Thus, if the same origin for the slide 14 was set, the operator can, by positioning the platform 51 and watching the coordinates change, find the same image which was previously recorded. The set X, Y function A26 must have been successfully performed previously in order for the X, Y function to be selected.

The clear function A30 will cause a purge of all the stored data images. After a clear function has been selected, the user will be asked to confirm the operation. To accept the confirmation, the user selects the yes key to confirm or, to reject the confirmation, the user selects the no key or presses the ESC key.

The focus function A32 provides color enhancement to the image so that the user can perform more precise focusing of the image. The system control 22 automatically provides different colors for gradations in grey level in the image. The operator then adjusts the focusing means of the microscope 15 until the object being focused on, for example an edge of the box, shows a clear color demarkation. This is an indication that the two separate levels or grey scale of the edge are in focus. This is much more difficult without color because the two grey levels may be close together and undiscernible without the color enhancement. The set light function A24 must have been successfully performed at least once in order to select the focus function. To restore the image to its original color, the focus function is selected a second time. If the color enhanced image is present when the user selects the measure function, the image will automatically be returned to its original color.

Selecting the analyze function A28 will change the display from the calibration screen to the analysis screen. The analysis screen provides a menu of functions shown in FIG. 17 that are necessary to perform the DNA measurements on the cellular material. Three criteria must be met in order to select the analyzed function. First, the set light function in the calibration screen must have been successfully performed at least once. Secondly, the calibration control cell count must be between 50 and 512, and finally the DNA conversion number must be greater than or equal to 1.0 and less than or equal to 99.99. The analyze function in the calibration menu works the same way as the analyze function in the main screen.

The analyze function options in the analysis menu are more fully shown in FIG. 17. The analysis menu allows the selection of a classify function A44, a focus function A46, a check light function A48, a select-2nd, function A50, an area 1-2 function A52, a scale function A54, a display X, Y function A56, a boundary function A58, a clear X, Y function A60, a help function A62, a report function A64 and a return to the main menu function A66.

The check light function A48 calculates the light level of the current image. The light level value is displayed on the analysis screen by the words "light level" in FIG. 13.

The select-2nd function A50 allows the user to select the second peak on the histogram displayed on the analysis screen. The mass, DNA index, and the area of the second peak are displayed on the screen under the words "second peak." The select-2nd function cannot be selected when the shown cell count is equal to zero. The shown cell count is displayed by the word "shown." After the select-2nd function has been selected, the cursor will move to a set of arrows and the current second peak location on the histogram will be highlighted in yellow. Initially the right most histogram data location is chosen as the second peak. Selecting the left arrow moves the second peak location to the left and the user selects the right arrow to move the second peak location to tie right. Every time an arrow is selected, the current peak data on the screen will be updated.

Below the histograms horizontal axis, one of three symbols will appear underneath the second peak location. A "less than" symbol will appear if the second peak lies in area one. A "greater than" symbol will appear if the second peak lies in area two. An up arrow symbol will appear if the second peak lies in neither area one nor area two. The reason for the three symbols is so that the second peak location can be identified after the select-2nd operation is exited. The vertical yellow line disappears once the select-2nd function is exited. The users presses the ESC key to exit the select second operation. The second peak data will also be automatically cleared when one of the following analysis screen functions is selected: clear, report, scale, or main.

The classify function A44 allows the user to classify the cells or objects in the current image. After the classify function has been selected, the user will be asked to confirm the operation. To accept the confirmation, the user will select the yes key, and to reject the confirmation, the user will select the no key or press the ESC key. If the classification is confirmed, the camera acquisitional stop and the cursor will move by the words "classify operation." When the cursor is at this location, the user can specify the classify operations. The numeric lock is activated which enables these functions. As was the case in the measure function, a magenta colored box will be placed around a current cell and the operations allow the user to move this cell identifier through the image to identify and classify the cells therein.

The display X, Y function A56 will change the display from the analysis screen to the X, Y field coordinates screen (FIG. 14). The X, Y field coordinate screen will display the X, Y coordinates of the first 512 images that are classified and stored. Also, the screen contains the functions that allow the sorting of the image fields by coordinates. The set X, Y function in the calibration screen must have been successfully performed before the display X, Y function is selected.

The X, Y field coordinate screen has several functions. One of the functions, "nearest" sorts the X, Y coordinates according to the distance from the current X, Y field position. The X function will sort the X, Y coordinates according to the X coordinate value. If there is a tie, then the Y coordinate value will determine the sort order. Similarly the Y function will sort the X, Y coordinates according to the Y coordinate value. If there is a tie, then X coordinate value will determine the sort order. The "field#" function will sort the X, Y coordinates according to the coordinates field number. The field number is the order in which the images were classified.

The page up function allows the user to display the previous page of X, Y coordinates, if any, and the page down function allows the use to display the next page of X, Y coordinates, if any. The exit function changes the display from the X, Y field coordinate screen to the analysis screen. Pressing the escape key is the same as selecting the exit function.

Selecting the X, Y function displays the X, Y coordinates of the current field. The coordinates will be continuously displayed until the user presses a key (except CTRL, ALT, and shift). The set X, Y function in the calibration screen must have successfully been performed before the X, Y function is selected. The X, Y function in the X, Y field coordinate screen works the same way as the X, Y function in the calibration and analysis screens.

The clear function A60 will clear all analysis related areas of data. After the clear function has been selected, the user will be asked to confirm the clear operation. To accept the confirmation, the user selects the yes key, or to reject the confirmation, the user will select the no key or press the ESC key.

The focus function A46 provides color enhancement to the image so that the user can perform more precise focusing of the image. The focus function in the analysis screen works the same way as the focus function in the calibration screen previously described.

The area 1-2 function A52 allows the user to specify two areas in the histogram displayed in the analysis screen. The purpose of this function is to identify the cell counts in certain areas in the histogram. The area 1-2 function cannot be selected when the shown cell count is equal to zero. The cell counts are displayed at the lower right portion of the screen. After area 1-2 is selected, the cursor will move to a row of

numbers that is below the histograms horizontal axis. The row of numbers allows the user to specify the locations of area 1 and area 2. The user types a "1" to specify that the current histogram position belongs to area 1. The user types a "2" to specify that the position belongs to area 2. The user types a "0" to specify the current histogram position belongs to neither area 1 or 2. The user is allowed to specify an area

5   1 without an area 2, but cannot specify an area 2 without an area 1. When both areas are specified, area 1 must be specified to the left of area 2. The area must be specified as continuous. To exit the area 1-2 function, the user presses the enter or ESC keys. If the user presses the enter key, area 1 of the histogram will be highlighted in green and the area 2 will be highlighted in magenta. The area cell counts will also be displayed. Pressing the ESC key will cause the program to disregard any of the changes that were made.

10   Area 1 and area 2 data will automatically be cleared when one of the following functions is selected: classify, clear, reports, scale or main.

The analysis function A42 is more fully described with respect to FIGS. 20 and 21. The operator will select a number of field locations 360, 361, and 362 in the slide specimen area 61 for analysis. The operator will move the X and Y knobs 110 and 17 for the microscope stage 51 to move into view on the

15   monitor screen 37 a first field of specimen cell objects to be analyzed for DNA content as well as for cell morphology if desired (FIG. 20). The program will place a box, for example at 300, over a particular specimen cell object 12 being displayed on the monitor 37 and then the operator will use a key to cause the scanning of the pixels (picture elements) of the specimen object to classify the cell in a manner similar to that disclosed in U.S. Patent 4,453,266 to give summed optical density for the cell specimen object i.e., a

20   stained cell nucleus, as well as its area, its roundness, and other classification information. Also, the operator has on the keyboard 36 several cell classification keys to be manually operated and the operator depresses one of the keys of a known category such as a type 0 normal cell; a type 1 cancer cell; a type 2 cancer cell; a type 3 cancer cell; and etc. On the monitoring screen 62 there will be an analysis histogram displaying the DNA content of the cells in the field. The operator selects a number of cells in each field or

25   area and then moves the microscope stage to position a number of different fields of specimen cells into view and takes and analyses a number of these specimen cells until the operator feels he has enough cells for a representative sample.

A histogram, such as shown will at this time be displayed on the monitor screen 62 which shows the number of cells of a particular DNA content and shows the DNA content averages for each of the reference

30   peaks, such as shown in FIG. 13. By depressing a print key, on the keyboard 36 the operator may print out the histogram shown in FIG. 13 on the printer 38. The data for the specimen cells is also stored internally within the system control 22 for later recall and comparison with data of any new specimen from the same patient for analysis relating to the progress or regression of a patient.

The operation of the analysis function will be now more fully described with respect to FIGS. 19 and 20

35   whereas there is shown a visual field which has been previously stored in the instrument. The field contains a number of cell objects which are to be classified and measured as to DNA content. When the program initially comes into this mode of operation, the first object in the field will be identified by scanning the pixels of the field in a raster like manner until a cell object is recognized. Once a cell object is recognized, an identification means such as box 300 is drawn around the object. This provides a visual identifier for the

40   operator to determine which cell object is presently being measured. In addition to the measurement, the operator has a number of options in the analysis menu. The primary option that an operator has is to classify a current object in block A202. He accomplishes this by pressing one of the numeric keys 0 through 5 which automatically puts the cell object of the identifying box 300 into the classification 0-5 selected. If the object identified is debris, not an abnormal cell, or not an identifiable cell object, the

45   operator can reject the current object by selecting a 9 on the keyboard as indicated in block A204.

After the classification or rejection of the object in box 300, the operator can move the identification box to the next unmeasured object as determined in block A208. A operator accomplishes this by pressing the keys CTRL/F2 which causes the program to erase the box 300 and search for the next identifiable cell object. This cell object is found and then another identifying box 302 is drawn around it to indicate to the

50   operator the function has been accomplished. In this manner, the entire group of cell objects can be classified and measured or rejected by repeating this process. FIG. 20 illustrates the procedure of scanning for a cell object, putting an identifying box around it, and the classifying or rejecting the object. In this manner, the program steps through the analysis procedure from object 300 to 302, 304, 306, 308, 310, 312, etc.

55   Further, if one of the cell objects to be classified does not look like the operator thinks it should and cannot be put in one of the previous classifications, or for some other reason the operator believes he has classified a previous object by mistake, then in block A206 by pressing CTRL/F1, he can move the identifying box back to the previously measured object. After identifying all cell objects in the particular field

being displayed, the operator has the option of going to another field by manipulating the X, Y positioning mechanism to provide more cells for the particular analysis.

When the operator has determined that enough cell objects had been analyzed, he may either terminate the analysis function by pressing either the enter key or escape key. If he terminates the analysis function by pressing the enter key, as indicated in block A212, then the data assembled for each of the measurements will be saved. However, if the analysis function is terminated by pressing the ESC key, then in block A216 the data will not be saved.

The report function A64 allows the user to specify which cell classifications are to be included in the histogram shown on the screen of the monitor 62. After the report function has been selected, the cursor can be moved to a option list which will allow the operator to specify the cell types. The following table specifies which key the operator presses in order to select a particular cell type.

CELL TYPE	KEY
normal	0 or n
1	1
2	2
3	3
4	4
lymphocyte	5 or L

Any combination of the types for the report data is allowed. The program will ignore any other characters than those listed in the table. The operator exits the report operation by pressing the enter or escape key. If the operator presses the enter key, he will change the types in the histogram to those which were specified. However, if the escape key is selected, the program will ignore any changes that were made and return normally. The functions area one and area two and the second peak data will automatically be cleared when the report operation is performed.

The scale function A54 allows the operator to change the scale of the horizontal axis of the histogram provided on the analysis screen. There are three scales to choose from, 0-16, 0-32, and 0-64. If the scale function is selected when the current scale is 0-16, then the new scale will be 0-32. If the scale function is selected when the current scale is 0-32, then the new scale will be 0-64. Likewise, if the scale function is selected when the current scale is 0-64, then the new scale will be 0-16. In this function the area 1, area 2, and second peak data will automatically be cleared when the scale operation is performed.

The boundary function A58 will change the display on monitor 37 from the analysis screen to the adjust boundary screen. The adjust boundary screen contains functions that are necessary to change the cell boundary, i.e., threshold. While addressing the boundary screen, the camera image acquisition will be halted.

The step function allows the operator to change the amount by which the boundary will change when one of the arrow keys is selected. The value must be in the range of 0-128. After the step size is selected, the cursor will move to the location on the screen where the user can type in a new step size value. To exit the step size function, the enter or escape keys are used. Pressing the enter key will save the step size change where pressing the escape key will ignore any change that was made. Initially, the step size value is equal to one.

The up arrow function will increase the cell boundary by the value of the step size and the down area function will decrease the cell boundary by the value of the step size. The exit function changes the display from the address boundary screen to the analysis screen. Pressing the escape key is the same as selecting the exit function.

In general, an interactive data collection and analysis scheme is used by the apparatus for the collection of specific parameters for both the calibration cell objects and the specimen cell objects. Each field which is selected is displayed on the monitor 37 and either the measure operation of the calibration screen or the classify operation of the analysis screen is chosen.

A software flow chart of a subroutine providing the interactive operations for the calibrate key operations and the analysis key operations, FIGS. 18 and 19, is illustrated in FIG. 24. When the operator selects either the measure operations or the classify operations, this program is called to provide the selection process for both the calibration cell objects and the specimen cell objects. The program begins in block A300 by performing a raster scan of the stored image pixel by pixel until it finds a pixel greater than the threshold value. The test for this operation is performed in block A302. If no pixel is found which is greater than the threshold, block A304 determines if the scan is complete. If not, the program loops back to block A300

where the scan is continued until all pixels in the image field are tested. After all pixels have been tested, the scan parameters are reset in block A308 and the cell object array updated in block A310.

At the time an image pixel is determined to be greater than the threshold, the program will label the object in block A306. The operation of labelling will now be more particularly described. The individualized cell objects in the digitized image are located by a scene analysis technique in which the raster scan is made of the digitized image to locate any pixel above the critical threshold. The technique then performs a four neighbor analysis of adjacent pixel elements and continues in a recursive manner locating "neighbors of the neighbors" which are above the threshold until the entire region of a cell object is defined. This technique is preferred to other scene analysis techniques, such as local boundary found from a gradient image, because it is fool proof in distinguishing the true region of a cell, particularly those cells having irregular or spiculed projections.

The four pixels (top, bottom, right side, and left side) surrounding the initially located pixel which are contiguous therewith are examined sequentially to identify the next pixel with a optical density or gray level value above the threshold. For instance, if the pixel located above the first pixel is not above the threshold, it is discarded from the labelling routine. The next pixel (right side) in a clockwise direction is then examined and may be above the threshold. If so, that pixel is then identified and stored in memory with the pixel as being a portion of the region of a cell. Next the address and density of the pixel found is stored in a pushdown list and the four neighboring pixels of that pixel are examined in the same clockwise order. This continues in a recursive manner until no neighbors are found above a threshold for a particular pixel. At this point the prior pixels on the pushdown list are reexamined to continue the neighbor search process until the entire number of pixels defining a region, i.e., the cell object has been identified. Thus, each of the pixels above the threshold of the region are identified and a complete enclosed region has been defined for a cell.

Once a cell object is labelled, a cell object table (FIG. 24C) is set up for the object as shown. The table lists the address of its entry point pixel, the number of pixels in the object, the X, Y points for the minimum and maximum points of the object, a count of the pixels in the perimeter of the object, the sum of the optical density of the object pixels, any classification provided for the object, and the X, Y coordinates of the field to which the object belongs. A plurality of the cell object tables comprise a temporary array, called a field array and shown in FIG. 24A, which is used to store the interactive data developed for the present field image under consideration.

In block A312 the program determines whether an automatic flag has previously been set. If so, the program will branch immediately to block A316 or, if not, negatively branch to block A313. Next, in block A313, a box or identifying border is placed around the object using the X, Y limits. This mode identifies a particular object in the field for the operator. In block A314, a key handler is entered to obtain a key press from the operator to determine which of the key functions of FIGS. 18 or 19 are to be accomplished. The key handler further determines which operation, either for calibration or analysis, is to be performed and only those keys which are associated with the present mode are enabled, all others are locked out. Once a key has been obtained, blocks A326-A342 will determine which function was selected and the progress of the routine.

Keys 0-5 as detected in block A326 provide for the acceptance of a calibration object or the classification of a specimen object. If such key is detected, then an affirmative branch continues the program at block A316. In block A318 the object is relabelled and in block A320 the object is colored (red) to indicate to the operator that it has been accepted or classified. The operator classifies the cell objects into different categories based upon visual clues such as morphology. The cells for analysis can be classified into a normal class 0, or one of several abnormal classes 1-5. The data class of the object is stored to its place in the associated object data table in block A316. Calibration objects are classified as type 0 or normal. The program then returns to the block A300 where the image scan registers are incremented to scan the field for the next object.

Alternatively, if the key press was a 9 as tested for in block A328, this means either a calibration cell object was rejected or a current specimen cell object was rejected. Thus, in block A322 the rejected cell object is colored in a different color (white) than an accepted or classified cell object, and the program returns to the scanning routine entry at block A300 to find another object. Coloring the cell object alerts the operator that the object has been analyzed in this field, coloring the object another color differentiates the object from an accepted or classified cell objects.

If, however, the key press is a CTRL/F1 as tested for in block A336, then the operator desires to move the identifying box to the last previously measured object. The program will then interrogate the field array to find the last object pointer in block A346. This pointer is used to create the box around the previous cell object by transferring control to block A313 before getting another key press in block A314. By using a series of CTRL/F1 keys the operator may selectively move the identifying box from previously measured

cell object to previously measured cell object in a reverse direction. If, after the box is placed around a particular cell object, the operator desires to reclassify that cell object, he then has the option of classifying it with the keys 0-5 in block A326.

5   The identifying box may be moved to the next unmeasured cell object by selecting the key CTRL/F2. The key is tested for in block A338 and if found, immediately returns the program control to the image scan entry in block A300. The effect of this operation is to allow the operator to skip the present cell object and move the identifying box to the next cell object without either rejecting or accepting the present cell. A series of CTRL/F2 presses will move the box forward through the cells without measuring them.

10   If all of the cell objects in a particular field appear normal as specimen cells, or as is generally the case with control cells they are acceptable, the operator may want to classify them all automatically. To accomplish this, an operator presses the key CTRL/F3. This key press is detected by block A339 and transfers control to block A341 where the automatic mode flag is set. The program then returns to the entry of the image scan in block A300. However, instead of going through the normal sequence of placing a box around the next object and waiting for a key press, the program will loop to automatically classify the rest of 15 the cells of a field. The automatic mode flag being set is sensed in block A313 and the program automatically transfers control to block A316. Automatically classified cells are categorized as normal or type 0. Thus, a scan and labelling loop will be executed via blocks A300, A302, A306, A313, A316, A318, and A320 until the scan of the entire field has been completed as sensed in block A304.

20   Another option that the operator can select is the cell cutting function which is entered by pressing the key CTRL/F4. This key is detected in block A342 and transfers control to the cell cutting function operation in block A352. When the CTRL and F4 keys are pressed, the user enters the cell cutting mode. While in this mode, the user is permitted to make cut lines inside the indentifier box. The operator cannot make a cut line over a pixel that belongs to a measured or a rejected cell. A measured cell is a cell that has been classified as type 0, 1, 2, 3, 4, or 5. Numeric lock must be activated in order to perform a cell cutting 25 operation. A cross hair is located where the cut is to take place. The following table lists the cell cutting operations that can be performed plus the key that must be pressed in order to select the desired operation. The function allows the splitting of overlapping cells by artificially making a perimeter between two areas, a cut. Thus, the labelling routine will only label one area as a cell object.

30

(KEY)	(ACTION)
0	Turn splitting on and off
1	Go down and left one step
2	Go down one step
3	Go down and right one step
4	Go left one step
5	Go to the center of the box
6	Go right one step
7	Go up and left one step
8	Go up one step
9	Go up and right one step
ENTER	Re-do last step (up to 100 pixels)
ESC	Exit cell splitting mode

45   A step is three pixels. When beginning a new cut, the first pixel will not be cut. For operation 5, the cross hair will not move if the center pixel belongs to a measured or rejected cell.

   After the cell cutting is performed in block A352, the scanner registers are set to the entry point of the particular object cut. The program then returns to the scan entry in block A300. Because the cell object has the same entry point but a different perimeter, the labelling routine (block A306) will label the cell object as 50 now cut.

   Another option that the operator has is the ability to select any object within a field. The selection of this mode is accomplished by pressing the CTRL/F5 key which is sensed in block A340. An affirmative branch from block A340 transfers control to block A348 where the select object mode is entered.

55   When the CTRL and F5 keys are pressed, the user enters the selection mode. Numeric lock must be activated in order to perform a selection operation. A cross hair will appear at the current selection point. The following table lists the selection operations that can be performed plus the key that must be pressed in order to select the desired operation.

18

(KEY)	(ACTION)
0	Select cross hair movement step size [5 or 15]
1	Go down and left one step
2	Go down one step
3	Go down and right one step
4	Go left one step
5	Go to the center of the image
6	Go right one step
7	Go up and left one step
8	Go up one step
9	Go up and right one step
ESC	Exit selection mode

When the selection mode is exited, the box will move to the first unmeasured cell after the selection point. If there are no cells after the cross hair, the box will go to the next unclassified cell.

After the object is selected by the above described technique, the scanner registers are set to the entry point of that particular object in block A348 and the program returns to the scan entry in block A300. This creates the identifier box around the object using its X, Y limits and provides the operator with the option of then pressing another key and performing other measurements and classifications on that selected object.

Another function is provided by key CTRL/F6 which is tested for in block A330. This feature provides an operator with the ability to move the indentifier box forward by reading the next cell object pointed in block A324 and then drawing around the box the chosen object in block A313. The key CTRL/F1, CTRL/F2 thereby allows an operator to quickly revise pervious cell classification by stepping forward and backwards, respectively, through the pointers of the previously measured cells.

When the enter key is sensed, in block A332, control of the program is transferred to block A310. In that block, the cell object array (FIG. 248) is updated with the present field array to store all of the data collected for the particular objects in the field. Alternatively, the sensing of the escape key returns the program immediately to the place in the software where it was called.

It will be appreciated that the illustrated control 22 has been programmed to do the cell classification and optical density analysis. Such classification and analysis is similar to that outlined in U.S. patent 4,453,266 for the classification or red blood cells and the present invention can be particularly useful in the analysis of red blood cells wherein the optical density of the hemoglobin content is measured rather than the DNA content as above described. As common in red blood cell analysis, the red blood cells need not be stained for image enhancement so that the staining calibration step may be eliminated for red blood cells when using the specific wave length of light specified in the aforementioned Bacus patents.

A further use of the present invention is to provide a precise measurement of hemoglobin in actual picograms for calibrating other instruments such as a Coulter counter. In such a process, the control blood cells 40 will have a known predetermined hemoglobin value and the specimen blood cells 12 of unknown hemoglobin value will be placed on the specimen area 61. Then the apparatus will be calibrated to show the histogram for the hemoglobin content of the specimen cells 12.

It will also be appreciated that the various calibration steps may be eliminated or combined and done simultaneously rather than done in the order and in the sequence and in the manner described for the preferred embodiment of the invention in making a DNA analysis. The use of the present invention for antigen analysis may include the steps of binding of monoclonal antibodies to the specimen and control cell objects. Later the monoclonal antibodies may be conjugated with an enzyme stain. Also, the monoclonal antibodies may be conjugated with a fluorescent material. Thereafter, the fluorescent stain may be excited at a wave length which excites the fluorescence and the specimen objects observed at another wave length at which fluorescent emission occurs. When the antigen is made for a particular virus, the control cell specimen objects may be treated with a nucleic acid probe specific for the genome of the virus.

Claims

1. A method of quantifying an amount of cellular material in an automatic image analyzing apparatus (11) including the steps of:

    providing a support means (14) which has calibration material (40) thereon, said calibration material having at least one parameter of a known value and having an optical density which varies in relation to staining concentrations;

providing specimen cell objects (12) having an unknown value for at least one parameter on a support means;

staining the specimen cell objects and calibration material at the same time with an image enhancing material;

calibrating (152) (A100) the automatic analyzing apparatus (11) to adjust for variations in the amount of stains on the specimen cell objects in response to analysis of the staining of the calibration material;

forming a digitized image from the stained specimen cells on a support;

analyzing (154) (A200) at least one parameter of said specimen cell objects using the digitized image thereof, said method being characterized by:

said calibration material having at least one parameter of a known value with said parameter correlating to an optical density which varies in relation to staining concentrations;

calibrating the automatic image analyzing apparatus for variations in light intensity (A24) by adjusting the apparatus in response to analysis of the intensity of light received by said image analyzing apparatus;

quantifying at least one parameter of said specimen cell objects using the digitized image thereof; and,

reporting (A64) on the analyzed specimen cell objects based on the quantification and analysis.

2. A method in accordance with Claim 1, characterized in that the calibration material is a stained reference cell object, and the known parameter value of said reference cell objects is a quantity of a cellular material, and the unknown parameter value of said specimen cell objects is a quantity of a cellular material, and in which the calibrating of the automatic image analysis apparatus (11) includes measuring the optical density of the stained reference cell object having a known quantity of a cellular material to determine a staining factor from the optical density of the stained reference cell object and using the staining factor and the measured optical density of the stained specimen cells objects (12) to quantify the amount of the stained cellular material in said specimen cell objects.

3. A method in accordance with Claims 1 or 2 characterized in that the step of calibrating the image analysis means for light variations includes:

illuminating with a light source (17a) the support means (14) containing a light calibration area (45) of known optical density;

analyzing the light intensity on the basis of distribution of light intensity of the light calibration area; and,

adjusting the light source in response to the distribution of light intensity.

4. A method in accordance with Claims 2 to 3, characterized in that said method includes the further steps of:

viewing (37) enlarged images of the specimen cell objects (12) in each of a plurality of successive fields;

selecting (A200) certain images of specimen cell objects (12) in the respective fields for image analysis;

gating (A326) the selected cell objects into a plurality of subpopulations on the basis of their viewed images; and,

analyzing (A106, A202) the selected cell objects for quantitative analysis and reporting.

5. A method in accordance with any of Claims 2 to 4, characterized in that the parameter quantified is the quantity of DNA in the specimen cell objects.

6. A method in accordance with Claims 1 to 5 characterized in that the step of calibrating the image analysis means of the image analysis apparatus (11) includes measuring (A106) reference cell objects for at least one parameter and relating this measurement to an actual mass number, said method including the further steps of:

generating at least one parameter distribution of the measured parameter for specimen cell objects; and

reporting (A64) the parameter distribution on a coordinate scale according to mass units.

7. A method in accordance with any of Claims 1 to 6, characterized in that the method includes the step of measuring the DNA mass of specimen cell objects and generating at least one parameter distribution of DNA mass showing a first peak and a second peak with a calculated parameter distribution for the first peak and a second peak; and

observing the location of and selecting (A50) the second peak for use in calculating at least one parameter distribution number for the second peak.

8. An apparatus for analyzing cell objects in an automatic image analyzing apparatus (11) including:

means for supporting (14) calibration material (40) and specimen cell objects (12), said specimen cell objects (12) having an unknown value for at least one parameter;

means for calibrating (152) (A100) the automatic analyzing apparatus (11) to adjust for variations in the amount of staining of specimen cell objects in response to analysis of the staining of the calibration material, said specimen cell objects and calibration material having been stained at the same time with an image enhancing material;

means for forming a digitized image from the stained specimen cells objects on a support;

means for analyzing (154) (A200) at least one parameter of said specimen cell objects using the digitized image thereof; and

means for reporting (A64) on the analyzed specimen cell objects based on the quantification and analysis, said apparatus characterized by:

said calibration material having at least one parameter of a known value with said parameter value correlating to an optical density which varies in relation to staining concentrations; and,

means for calibrating the automatic image analyzing apparatus for variations in light intensity by adjusting the apparatus in response to analysis of the intensity of light received by said image analyzing apparatus; and

means for quantifying at least one parameter of said specimen cell objects using the digitized image thereof

9. An apparatus in accordance with Claim 8, characterized in that the calibration material is a stained reference cell object, the known parameter value of said reference cell objects is a quantity of a cellular material, and the unknown parameter value of said specimen cell objects is a quantity of a cellular material, and in which said means for calibrating the automatic image analyzing apparatus (11) includes:

means for measuring the optical density of the stained reference cell object having a known quantity of a cellular material to determine a staining factor from the optical density of the stained reference cell objects, and,

means for quantifying the amount of the stained cellular material in said specimen cell objects based on the staining factor and the measured optical density of the stained specimen cells objects (12).

10. An apparatus in accordance with Claims 8 or 9 characterized in that the means for calibrating the image analysis means for light variations includes:

means for illuminating (17a) the support means (14) containing a light calibration area (45);

means for analyzing the light intensity received by the image analysis means on the basis of distribution of light intensity of the light calibration area; and

means for adjusting the light source in response to the distribution of light intensity.

11. An apparatus in accordance with Claims 8 to 10, characterized in that said apparatus further comprises:

means for viewing (37) enlarged images of the specimen cell objects (12) in each of a plurality of successive fields;

means for selecting (A200) certain images of specimen cell objects (12) in the respective fields for image analysis;

means for gating (A326) the selected cell objects into a plurality of subpopulations on the basis of their viewed images; and

means for analyzing (A106, A202) the selected cell objects for quantitative analysis and reporting.

12. An apparatus in accordance with Claims 9 to 11 characterized in that the means for calibrating th image analysis apparatus (11) includes means for measuring (A106) reference cell objects for at least one parameter and relating this measurement to an actual mass number, said apparatus including:

means for generating at least one parameter distribution of the measured parameter for specimen cell objects; and

means for reporting (A64) the parameter distribution on a coordinate scale according to mass units.

5 **13.** An apparatus in accordance with any of Claim 12, characterized in that the apparatus for reporting the DNA mass of specimen cell objects includes:

means for generating at least one parameter distribution of DNA mass showing a first peak and a second peak with a calculated parameter distribution for the first peak and a second peak; and

means for observing the location of and selecting (A50) the second peak for use in calculating at
10 least one parameter distribution number for the second peak.

Patentansprüche

1. Verfahren zur Quantifizierung einer Menge von zellularem Material in einem automatischen Bildanalyse-
15 gerät (11) mit folgenden Schritten:

Bereitstellung von Trägermitteln (14) mit darauf befindlichem Eichmaterial (40), das wenigstens einen Parameter mit bekanntem Wert und eine sich in Abhängigkeit von Farbekonzentrationen ändernde optische Dichte besitzt;

Bereitstellung von Probezellenobjekten (12) mit unbekanntem Wert wenigstens eines Parameters auf
20 den Trägermitteln;

gleichzeitige Einfärbung der Probezellenobjekte und des Eichmaterials mit einem Bildverstärkungsma- terial;

Eichen (152) (A100) des automatischen Analysegerätes (11) zur Einjustierung hinsichtlich von Änderun- gen von Farbstoffen auf den Probezellenobjekten in Abhängigkeit von der Analyse des Färbens des
25 Eichmaterials;

Bildung eines digitalisierten Bildes aus den gefärbten Probezellen auf einem Träger;

Analyse (154) (A200) wenigstens eines Parameters der Probezellenobjekte unter Verwendung von deren digitalisiertem Bild, **dadurch gekennzeichnet, daß**

das Eichmaterial wenigstens einen Parameter mit bekanntem Wert besitzt, welcher mit der sich in
30 Abhängigkeit von Färbekonzentrationen ändernden optischen Dichte korreliert ist;

das automatische Bildanalysegerät hinsichtlich Änderungen der Lichtintensität (A24) durch Einjustieren des Gerätes in Abhängigkeit von der Analyse der durch das Bildanalysegerät empfangenen Lichtinten- sität geeicht wird;

wenigstens ein Parameter der Probezellenobjekte unter Verwendung von deren digitalisiertem Bild
35 quantifiziert wird; und

die analysierten Probezellenobjekte auf der Basis der Quantifizierung und Analyse gemeldet werden (A64).

2. Verfahren nach Anspruch 1, **dadurch gekennzeichnet**, daß das Eichmaterial ein gefärbtes Bezugszel-
40 lenobjekt, der bekannte Parameterwert der Bezugszellenobjekte eine Menge eines zellularen Materials und der unbekannte Parameterwert der Probezellenobjekte eine Menge eines zellularen Materials ist und in dem die Eichung des automatischen Bildanalysegerätes (11) die Messung der optischen Dichte des eine bekannte Menge an zellularem Material besitzenden gefärbten Bezugszellenobjektes zwecks Bestimmung eines Färbungsfaktors aus der optischen Dichte des gefärbten Bezugszellenobjektes und
45 Verwendung des Färbungsfaktors und der gemessenen optischen Dichte der gefärbten Probezellenob- jekte (12) zwecks Quantifizierung der Menge des gefärbten zellularen Materials in den Probezellenob- jekten enthält.

3. Verfahren nach Anspruch 1 oder 2, **dadurch gekennzeichnet**, daß der Schritt des Eichens der
50 Bildanalysemittel hinsichtlich Lichtänderungen: die Beleuchtung der einen Lichteichbereich (45) be- kannter optischer Dichte enthaltenen Trägermittel mit einer Lichtquelle (17a);

die Analyse der Lichtintensität auf der Basis der Lichtintensitätsverteilung des Lichteichbereiches; und die Einjustierung der Lichtquelle in Abhängigkeit von der Lichtintensitätsverteilung enthält.

55
4. Verfahren nach Anspruch 2 oder 3, **dadurch gekennzeichnet**, daß es die weiteren Schritte:

eine Betrachtung (37) vergrößerter Bilder der Probezellenobjekte (12) in jedem Feld einer Vielzahl von aufeinanderfolgenden Feldern;

eine Auswahl (A200) bestimmter Bilder von Probezellenobjekten (12) in entsprechenden Feldern zur Bildanalyse;

eine Trennung (A326) der ausgewählten Zellenobjekte in eine Vielzahl von Unterpopulationen auf der Basis ihrer betrachten Bilder; und

5   eine Analyse (A106, A202) der ausgewählten Zellenobjekte zur quantitativen Analyse und Meldung enthält.

5.   Verfahren nach den Ansprüchen 2 bis 4, **dadurch gekennzeichnet,** daß der quantifizierte Parameter die DNA-Menge in den Probezellenobjekten ist.

10

6.   Verfahren nach den Ansprüchen 1 bis 5, **dadurch gekennzeichnet,** daß der Eichschritt der Bildanalyse des Bildanalysegerätes (11) eine Messung (A106) von Bezugszellenobjekten für wenigstens einen Parameter und in Beziehungsetzen dieser Messung zu einer tatsächlichen Massenzahl enthält und das Verfahren die weiteren Schritte:

15   • einer Erzeugung wenigstens einer Parameterverteilung des gemessenen Parameters für Probezellenobjekte; und

Meldung (A64) der Parameterverteilung auf einer Masseneinheiten entsprechenden Koordinatenskala enthält.

20   7.   Verfahren nach einem der Ansprüche 1 bis 6, **dadurch gekennzeichnet,** daß es weiterhin den Schritt einer Messung der DNA-Masse von Probezellenobjekten und Erzeugung wenigstens einer Parameterverteilung einer eine erste Spitze und eine zweite Spitze zeigenden DNA-Masse mit einer berechneten Parameterverteilung für die erste und die zweite Spitze; und

eine Beobachtung der Lage der zweiten Spitze und deren Auswahl (A50) zur Verwendung bei der 25   Berechnung wenigstens einer Parameterverteilungszahl für die zweite Spitze enthält.

8.   Vorrichtung zur Analyse von Zellenobjekten in einem automatischen Bildanalysegerät (11) enthaltend:

Trägermittel (14) für Eichmaterial (40) und Probezellenobjekte (12), die einen unbekannten Wert für 30   wenigstens einen Parameter besitzen;

Eichmittel (152) (A100) zur Eichung des automatischen Analaysegerätes (11) zwecks Einjustierung hinsichtlich Änderungen der Menge von gefärbten Probezellenobjekten in Abhängigkeit von einer Analyse der Färbung des Eichmaterials, wobei die Probezellenobjekte und das Eichmaterial gleichzeitig mit einem Bildverstärkungsmaterial eingefärbt werden;

35   Mittel zur Bildung eines digitalisierten Bildes aus den gefärbten Probezellenobjekten auf einem Träger;

Analysemittel (154) (A200) zur Analyse wenigstens eines Parameters der Probezellenobjekte unter Verwendung von deren digitalisiertem Bild; und

Meldemittel zum Melden (A64) über die analysierten Probezellenobjekte auf der Basis der Quantifizierung und Analyse, **dadurch gekennzeichnet,** daß

40   das Eichmaterial wenigstens einen Parameter bekannten Wertes besitzt, der mit einer sich in Abhängigkeit von den Färbekonzentrationen ändernden optischen Dichte korreliert ist; und

Mittel zur Eichung des automatischen Bildanalysegerätes hinsichtlich Änderungen der Lichtintensität durch Einjustierung des Gerätes in Abhängigkeit von der Analyse der durch das Bildanalysegerät empfangene Lichtintensität; und

45   Mittel zur Quantifizierung wenigstens eines Parameters der Probezellenobjekte unter Verwendung von deren digitalisiertem Bild vorgesehen sind.

9.   Vorrichtung nach Anspruch 8, **dadurch gekennzeichnet,** daß das Eichmaterial ein gefärbtes Bezugs- 50   zellenobjekt, der bekannte Parameterwert der Bezugszellenobjekte eine Menge von zellularem Material und der unbekannte Parameterwert der Bezugszellenobjekte eine Menge von zellularem Material ist und daß die Mittel zur Eichung des automatischen Bildanalysegerätes (11)

Mittel zur Messung der optischen Dichte des eine bekannte Menge von zellularem Material besitzenden gefärbten Bezugszellenobjektes zwecks Bestimmung eines Färbungsfaktors aus der optischen Dichte 55   der gefärbten Bezugszellenobjekte, und

Mittel zur Quantifizierung der Menge des gefärbten zellularen Materials in den Probezellenobjekten auf der Basis des Färbungsfaktors und der gemessenen optischen Dichte der gefärbten Probezellenobjekte (12)

enthalten.

10. Vorrichtung nach Anspruch 8 oder 9, **dadurch gekennzeichnet,** daß die Mittel zur Eichung der Bildanalysemittel hinsichtlich Lichtänderungen

5   Mittel zur Beleuchtung (17a) der einen Lichteichbereich (45) enthaltenden Trägermittel (14);
Mittel zur Analyse der durch die Bildanalysemittel empfangenen Lichtintensität auf der Basis der Lichtintensitätsverteilung des Lichteichbereiches; und
Mittel zur Einjustierung der Lichtquelle in Abhängigkeit von der Lichtintensitätsverteilung enthalten.

10

11. Vorrichtung nach den Ansprüchen 8 bis 10, **dadurch gekennzeichnet,** daß sie weiterhin
Mittel zur Betrachtung (37) vergrößerter Bilder der Probezellenobjekte (12) in jedem Feld einer Vielzahl von aufeinanderfolgenden Feldern;
Mittel zur Auswahl (A200) bestimmter Bilder von Probezellenobjekten (12) in entsprechenden Feldern

15   zur Bildanalyse;
Mittel zur Trennung (A326) der ausgewählten Zellenobjekte in eine Vielzahl von Unterpopulationen auf der Basis ihrer betrachteten Bilder; und
Mittel zur Analyse (A106, A202) der ausgewählten Zellenobjekte für eine quantitative Analyse und Meldung

20   umfaßt.

12. Vorrichtung nach den Ansprüchen 9 bis 11, **dadurch gekennzeichnet,** daß die Mittel zur Eichung des Bildanalysegerätes (11) Mittel zur Messung (A106) von Bezugszellenobjekten für wenigstens einen Parameter und Inbeziehungsetzen dieser Messung mit einer tatsächlichen Massenzahl enthalten und

25   daß die Vorrichtung
Mittel zur Erzeugung wenigstens einer Parameterverteilung des gemessenen Parameters für Probezellenobjekte; und
Mittel zur Meldung (A64) der Parameterverteilung auf einer Masseneinheiten entsprechenden Koordinatenskala

30   enthält.

13. Vorrichtung nach Anspruch 12, **dadurch gekennzeichnet,** daß die Einrichtung zur Meldung der DNA-Masse von Probezellenobjekten
Mittel zur Erzeugung wenigstens einer Parameterverteilung der eine erste und eine zweite Spitze

35   zeigenden DNA-Masse mit einer berechneten Parameterverteilung für die erste und zweite Spitze; und
Mittel zur Beobachtung der Lage der zweiten Spitze und deren Auswahl (A50) zur Verwendung bei der Berechnung wenigstens einer Parameterverteilungszahl für die zweite Spitze
enthält.

40 **R vendications**

1. Procédé de quantification d'une masse de matière cellulaire dans un appareil automatique (11) d'analyse d'images, comprenant les étapes qui consistent :
à utiliser un moyen (14) de support portant une matière (40) d'étalonnage, ladite matière d'étalon-

45   nage ayant au moins un paramètre d'une valeur connue et ayant une densité optique qui varie en fonction de concentrations de colorant ;
à placer des cellules-objets échantillons (12) ayant une valeur inconnue pour au moins un paramètre sur un moyen de support ;
à colorer les cellules-objets échantillons et la matière d'étalonnage, simultanément, avec une

50   matière de renforcement d'image ;
à étalonner (152) (A100) l'appareil d'analyse automatique (11) pour un réglage portant sur des variations de la quantité de colorants sur les cellules-objets échantillons en réponse à une analyse du colorant de la matière d'étalonnage ;
à former une image numérisée à partir des cellules échantillons colorées sur un support ;

55   à analyser (154) (A200) au moins un paramètre desdites cellules-objets échantillons en utilisant leur image numérisée, ledit procédé étant caractérisé en ce que :
ladite matière d'étalonnage possède au moins un paramètre d'une valeur connue, ledit paramètre étant en corrélation avec une densité optique qui varie en fonction des concentrations de colorants ;

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à étalonner l'appareil d'analyse automatique d'image pour des variations de l'intensité lumineuse (A24) en réglant l'appareil en réponse à une analyse de l'intensité de la lumière reçue par ledit appareil d'analyse d'images ;

à quantifier au moins un paramètre desdites cellules-objets échantillons en utilisant leur image numérisée ; et

à effectuer un rapport (A64) sur les cellules-objets échantillons analysées sur la base de la quantification et de l'analyse.

2. Procédé selon la revendication 1, caractérisé en ce que la matière d'étalonnage est une cellule-objet échantillon de référence colorée, et la valeur de paramètre connue desdites cellules-objets de référence est une quantité de matière cellulaire, et la valeur de paramètre inconnue desdites cellules-objets échantillons est une quantité de matière cellulaire, et dans lequel l'étalonnage de l'appareil d'analyse automatique (11) d'images comprend une mesure de la densité optique de la cellule-objet de référence colorée ayant une quantité connue de matière cellulaire pour déterminer un facteur de coloration à partir de la densité optique de la cellule-objet de référence colorée et l'utilisation du facteur de coloration et de la densité optique mesurée des cellules-objets échantillons colorés (12) pour quantifier la quantité de matière cellulaire colorée dans lesdites cellules-objets échantillons.

3. Procédé selon la revendication 1 ou 2, caractérisé en ce que l'étape d'étalonnage des moyens d'analyse d'image, portant sur des variations de la lumière, consiste :

à éclairer à l'aide d'une source de lumière (17a) le moyen de support (14) contenant une zone (45) d'étalonnage de la lumière d'une densité optique connue ;

à analyser l'intensité de la lumière sur la base de la distribution de l'intensité de la lumière de la zone d'étalonnage de la lumière ; et

à régler la source de lumière en réponse à la distribution de l'intensité de la lumière.

4. Procédé selon la revendication 2 ou 3, caractérisé en ce qu'il comprend les autres étapes qui consistent :

à observer (37) des images agrandies des cellules-objets échantillons (12) dans chacun de plusieurs champs successifs ;

à sélectionner (A200) certaines images de cellules-objets échantillons (12) dans les domaines respectifs pour une analyse d'image ;

à aiguiller (A326) les cellules-objets sélectionnées dans plusieurs sous-populations sur la base de leurs images observées ; et

à analyser (A106, A202) les cellules-objets sélectionnées pour une analyse quantitative et un rapport.

5. Procédé selon l'une quelconque des revendications 2 à 4, caractérisé en ce que le paramètre quantifié est la quantité d'ADN dans les cellules-objets échantillons.

6. Procédé selon les revendications 1 à 5, caractérisé en ce que l'étape d'étalonnage des moyens d'analyse d'images de l'appareil (11) d'analyse d'images comprend une mesure (A106) de cellules-objets de référence pour au moins un paramètre et la mise en relation de cette mesure avec un nombre de masse réel, ledit procédé comprenant les autres étapes qui consistent :

à générer au moins une distribution du paramètre mesuré pour des cellules-objets échantillons ; et

à reporter (A64) la distribution de paramètre sur une échelle de coordonnées en fonction d'unités de masse.

7. Procédé selon l'une quelconque des revendications 1 à 6, caractérisé en ce qu'il comprend l'étape qui consiste à mesurer la masse d'ADN de cellules-objets échantillons et à générer au moins une distribution de paramètre de la masse d'ADN montrant une première pointe et une seconde pointe avec une distribution de paramètre calculée pour la première pointe et une seconde pointe ; et

à observer la position de la seconde pointe et à sélectionner (A50) la seconde pointe pour l'utiliser dans le calcul d'au moins un nombre de distribution de paramètre pour la seconde pointe.

8. Appareil pour analyser des cellules-objets dans un appareil automatique (11) d'analyse d'images, comprenant :

un moyen (14) destiné à supporter une matière d'étalonnage (40) et des cellules-objets échantillons

(12), lesdites cellules-objets échantillons (12) ayant une valeur inconnue portant sur au moins un paramètre ;

des moyens (152) (A100) pour étalonner l'appareil (11) d'analyse automatique afin d'effectuer un réglage portant sur les variations de la quantité de coloration des cellules-objets échantillons en réponse à une analyse de la coloration de la matière d'étalonnage, lesdites cellules-objets échantillons et la matière d'étalonnage ayant été colorées simultanément avec une matière de renforcement d'image ;

des moyens pour former une image numérisée à partir des cellules-objets échantillons colorées sur un support ;

des moyens (154) (A200) pour analyser au moins un paramètre desdites cellules-objets échantillons en utilisant leur image numérisée ; et

des moyens (A64) pour effectuer un rapport portant sur les cellules-objets échantillons analysées sur la base de la quantification et de l'analyse, ledit appareil étant caractérisé en ce que :

ladite matière d'étalonnage possède au moins un paramètre d'une valeur connue, ladite valeur du paramètre étant en corrélation avec une densité optique qui varie en fonction de concentrations de coloration ; et

des moyens pour étalonner l'appareil automatique d'analyse d'image pour des variations de l'intensité de la lumière par réglage de l'appareil en réponse à une analyse de l'intensité de la lumière reçue par ledit appareil d'analyse d'images ; et

des moyens destinés à quantifier au moins un paramètre desdites cellules-objets échantillons en utilisant leur image numérisée.

9. Appareil selon la revendication 8, caractérisé en ce que la matière d'étalonnage est une cellule-objet de référence colorée, la valeur de paramètre connue desdites cellules-objets de référence est une quantité de matière cellulaire, et la valeur de paramètre inconnue desdites cellules-objets échantillons est une quantité de matière cellulaire, et dans lequel lesdits moyens pour étalonner l'appareil automatique (11) d'analyse d'images comprennent :

des moyens pour mesurer la densité optique de la cellule-objet de référence colorée ayant une quantité connue de matière cellulaire afin de déterminer un facteur de coloration à partir de la densité optique des cellules-objets de référence colorées, et

des moyens pour quantifier la quantité de matière cellulaire colorée dans lesdites cellules-objets échantillons sur la base du facteur de coloration et de la densité optique mesurée des cellules-objets échantillons colorées (12).

10. Appareil selon la revendication 8 ou 9, caractérisé en ce que les moyens pour étalonner les moyens d'analyse d'images en fonction de variations de la lumière comprennent :

des moyens (17a) pour éclairer le moyen de support (14) contenant une zone (45) d'étalonnage de la lumière ;

des moyens pour analyser l'intensité de la lumière reçue par les moyens d'analyse d'images sur la base d'une distribution de l'intensité de la lumière de la zone d'étalonnage de la lumière ; et

des moyens pour régler la source de lumière en réponse à la distribution de l'intensité de la lumière.

11. Appareil selon les revendications 8 à 10, caractérisé en ce qu'il comporte en outre :

des moyens (37) pour observer des images agrandies des cellules-objets échantillons (12) dans chacun de plusieurs champs successifs ;

des moyens (A200) pour sélectionner certaines images de cellules-objets échantillons (12) dans les champs respectifs pour une analyse d'images ;

des moyens (A326) pour diriger les cellules-objets sélectionnées vers plusieurs sous-populations sur la base de leurs images observées ; et

des moyens (A106, A202) pour analyser les cellules-objets sélectionnées pour une analyse quantitative et un rapport.

12. Appareil selon les revendications 9 à 11, caractérisé en ce que les moyens destinés à étalonner l'appareil (11) d'analyse d'images comprennent des moyens (A106) destinés à mesurer au moins un paramètre de cellules-objets de référence et à lier cette mesure à un nombre de masse réel, ledit appareil comprenant :

des moyens destinés à générer au moins une distribution du paramètre mesuré pour des cellules-

objets échantillons ; et

des moyens (A64) destinés à reporter la distribution de paramètre sur une échelle de coordonnées en fonction d'unités de masse.

5   13. Appareil selon la revendication 12, caractérisé en ce que l'appareil destiné à reporter la masse d'ADN de cellules-objets échantillons comprend :

des moyens destinés à générer au moins une distribution de paramètre de la masse d'ADN montrant une première pointe et une seconde pointe, avec une distribution de paramètre calculée pour la première pointe et une seconde pointe ; et

10   des moyens pour observer la position de la seconde pointe et pour sélectionner (A50) la seconde pointe pour une utilisation dans le calcul d'au moins un nombre de distribution de paramètre pour la seconde pointe.

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50

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FIG. 1

FIG. 2

FIG. 2A

| INSTRUMENT CONTROL LOGIC
IMAGE DISPLAY AND INSTRUCTION
DISPLAY CONTROL LOGIC |

| PATIENT LABELING |

| LIGHT CALIBRATION |

| CONTROL CELL CALIBRATION |

| CELL DATA ACQUISITION,
CELL CLASSIFICATION, &
CELL ANALYSIS |

| REPORT |

FIG. 3

FIG. 4

24 BITS

X

XCLK

100 MILLISECS

FIG. 5A

40

54

CAS

61

53

14

X

45

FIG. 5B

5C

12

14

40

CAS

54

61

53

52

45

5C

FIG. 5C


14
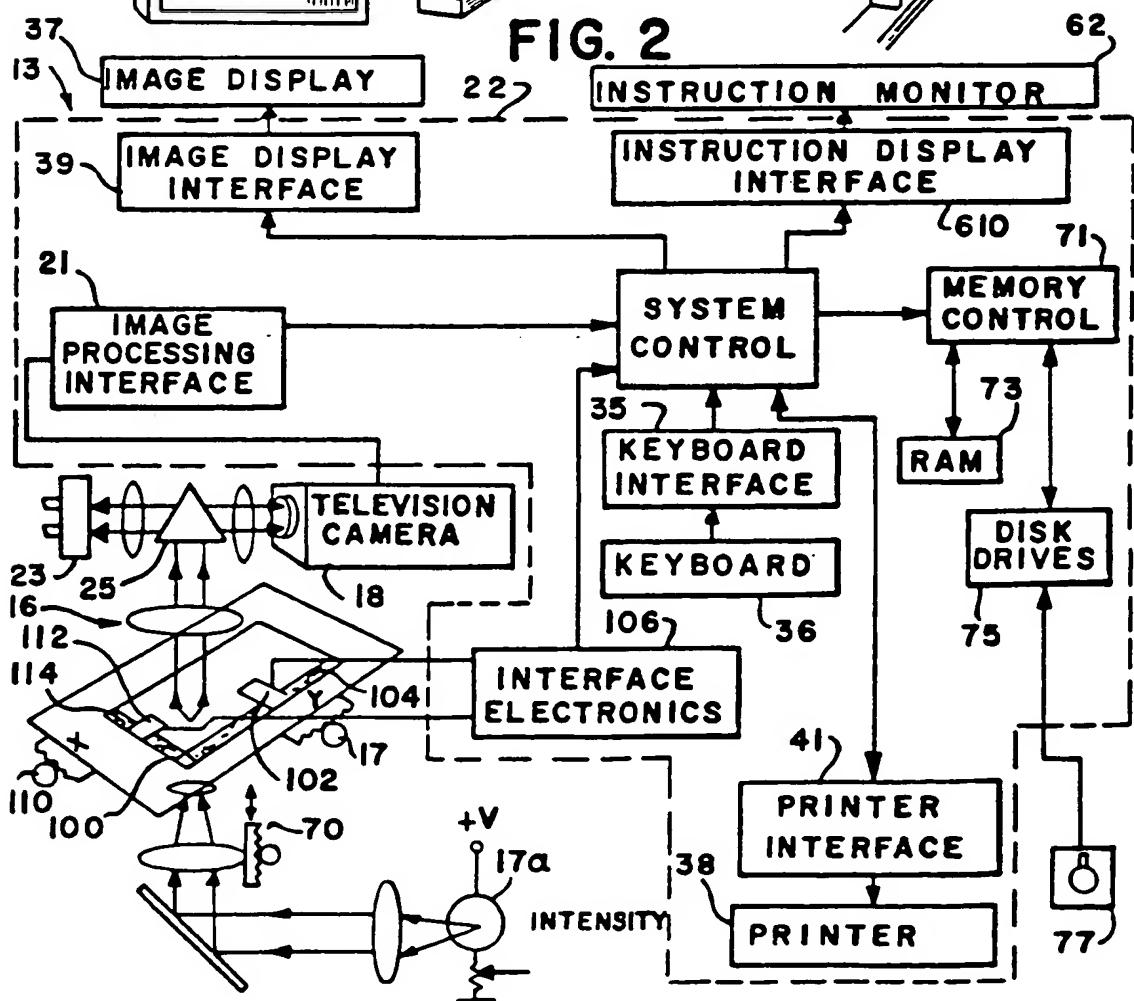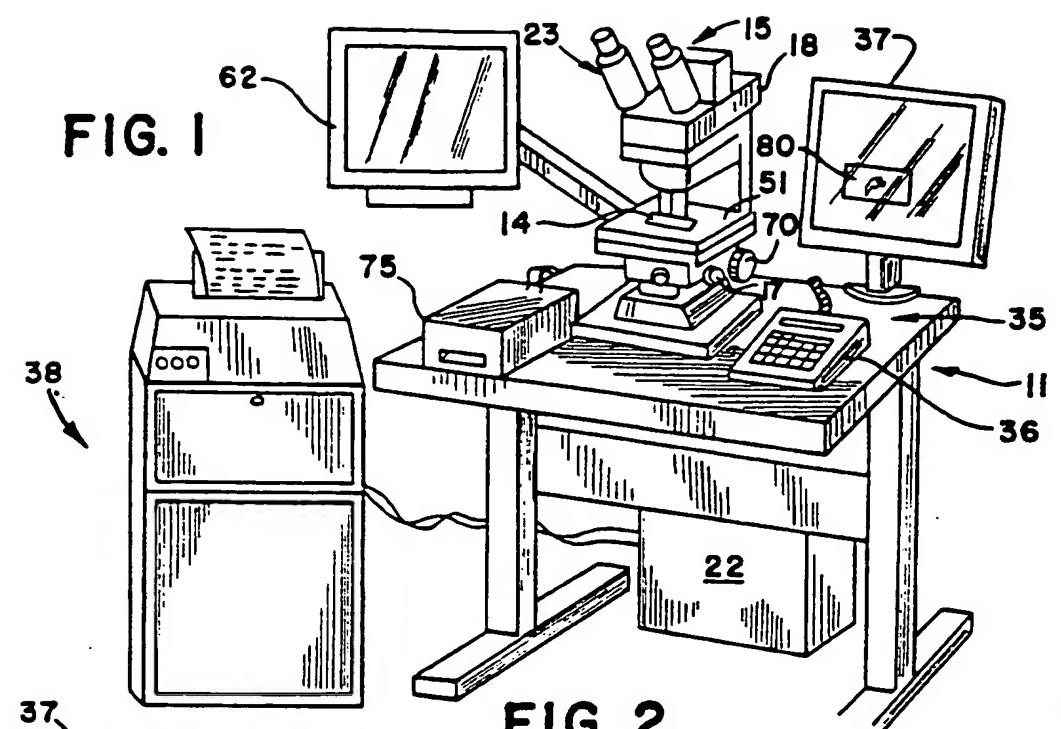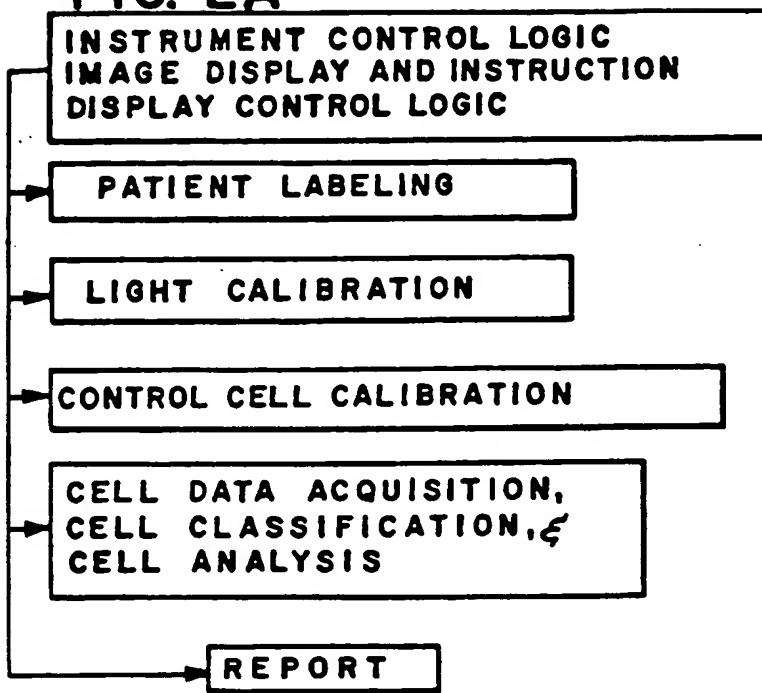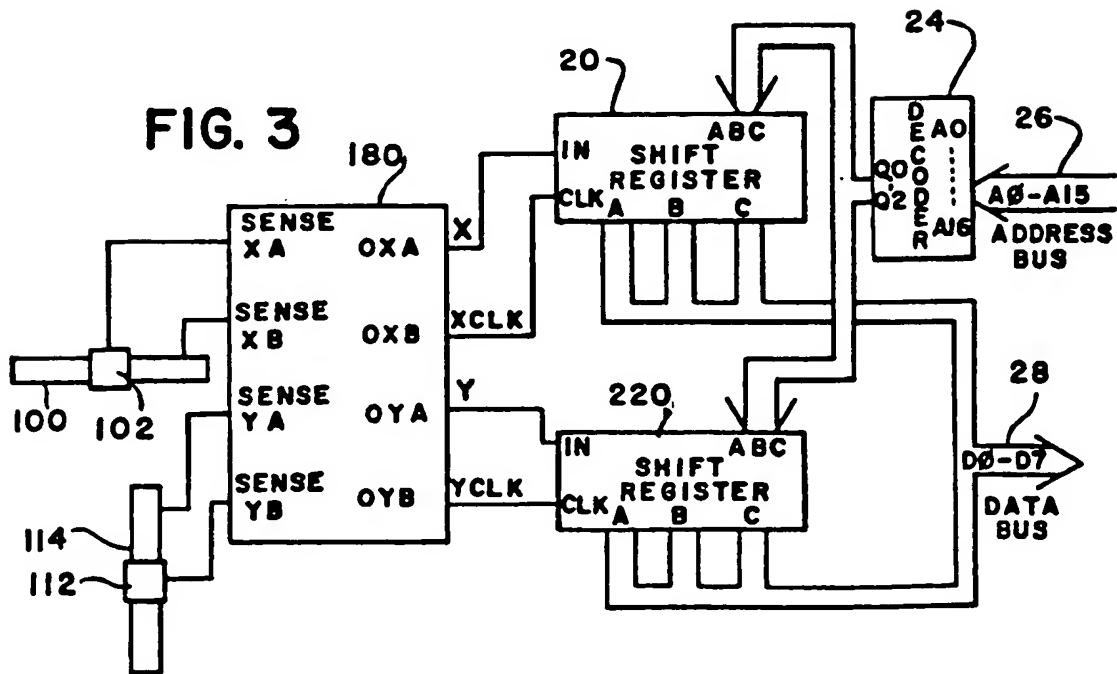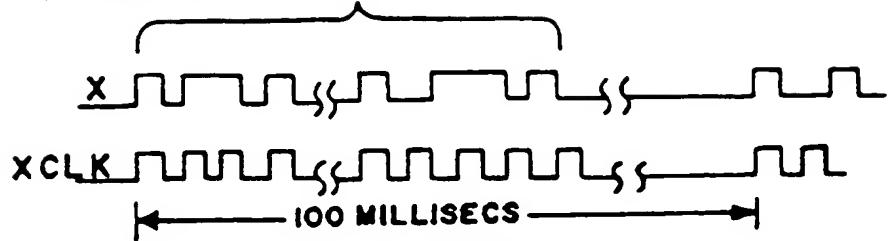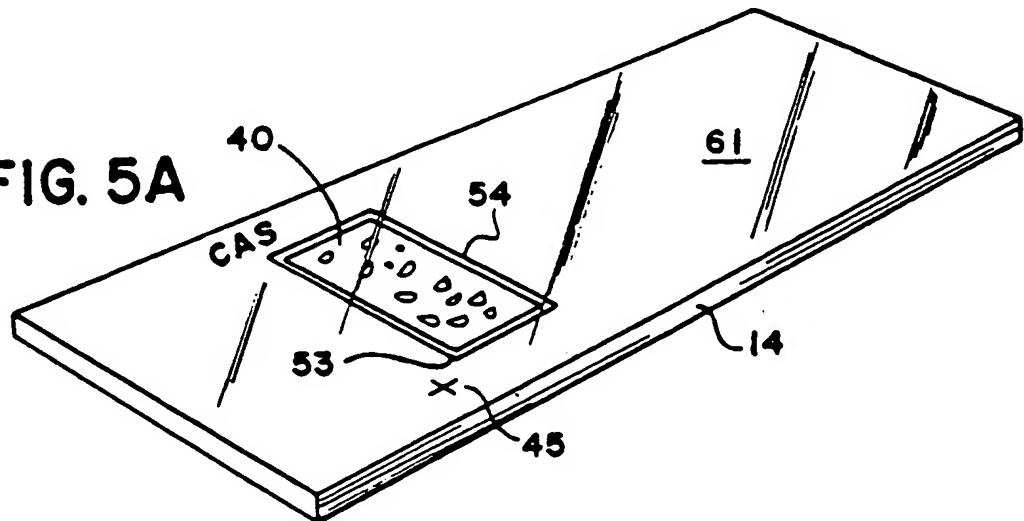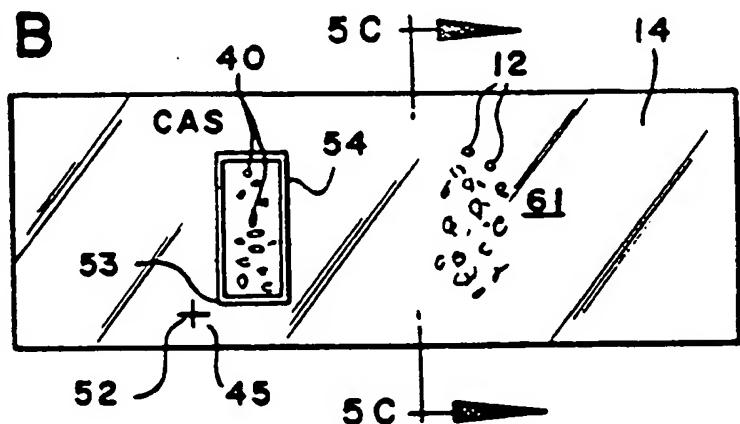
FIG. 6
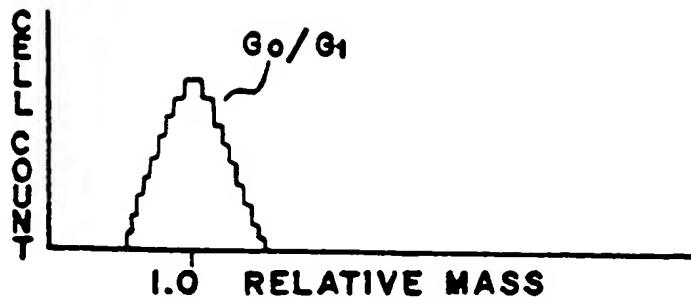
FIG. 7
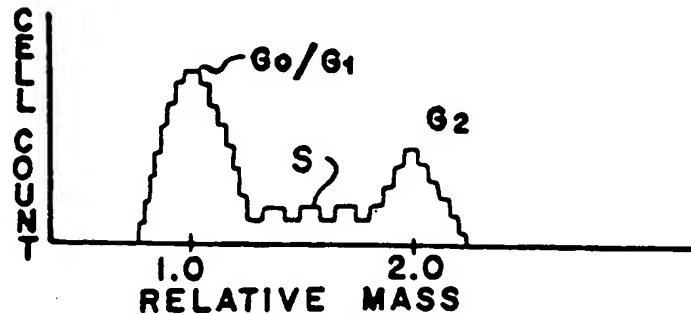
FIG. 8
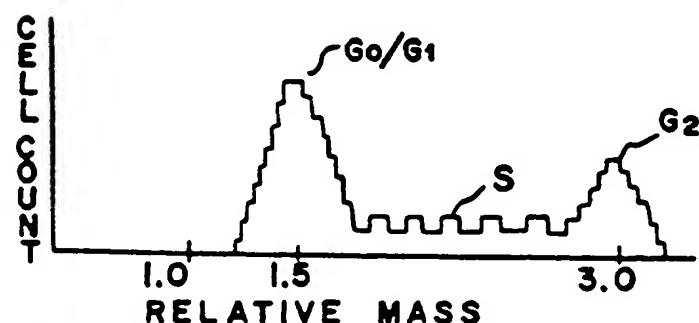
FIG. 9
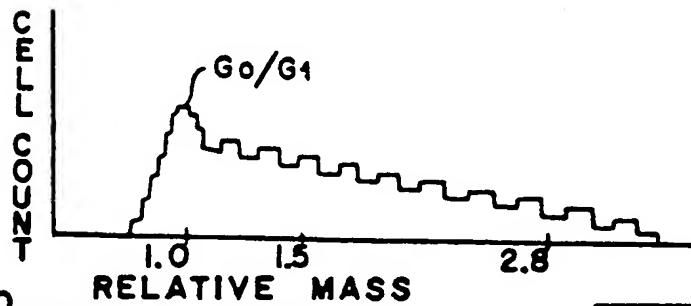

FIG. 10
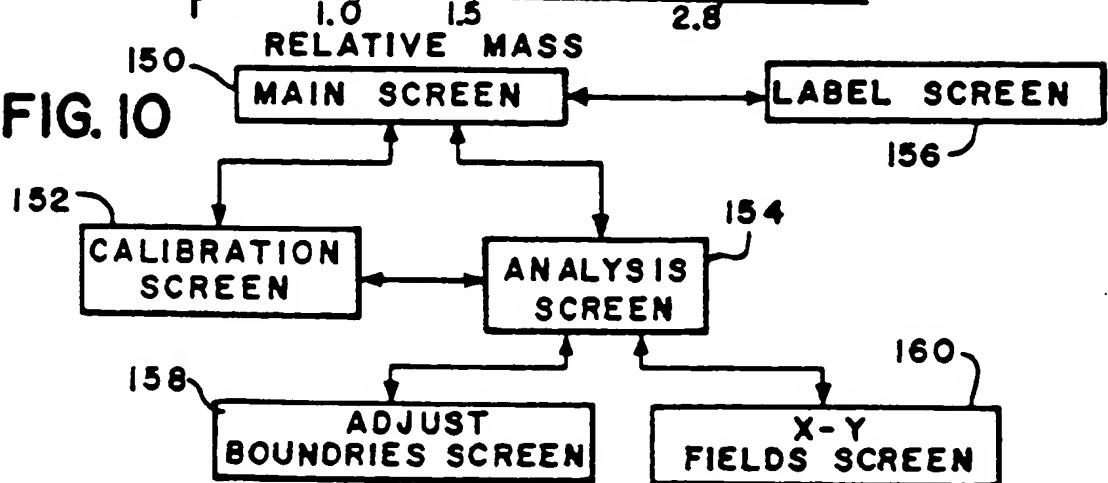
FIG. 11   MAIN SCREEN

PATIENT IDENTIFICATION
ACCESSION NUMBER
DNA INDEX BASED ON 7.18 PICOGRAMS

OPTION LIST

| CALIBRATION STATUS		ANALYSIS STATUS	
CELL COUNT	87	CELL COUNT	O
PEAK O.D. VALUE	8400	MAIN PEAK DNA	.O pg. AIO
LIGHT LEVEL	O	MAIN PEAK INDEX	.O
		2ND PEAK DNA	.O pg.
		2ND PEAK INDEX	.O

CONFIRM FUNCTION
YES
NO

FIG. 12   CALIBRATION SCREEN

PATIENT IDENTIFICATION
ACCESSION NUMBER
DNA INDEX BASED ON 7.18 PICOGRAMS

OPTION LIST

DISTRIBUTION OF CALIBRATION DNA

CELL COUNT
70
56
42
28
14
0

0   4000   8000   12000   16000

RELATIVE CELL MASS
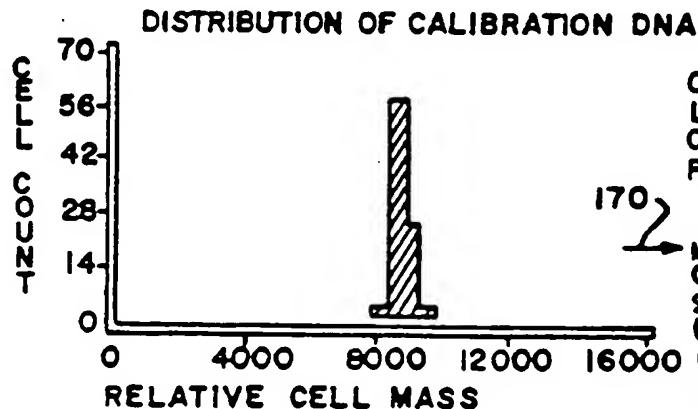
CALIBRATION DATA
LIGHT LEVEL   O
CELL COUNT   87
PEAK VALUE 8400

170

MEASURE OPERATIONS O
O= ACCEPT CELL
9= REJECT CELL
(CR)= STOP/SAVE DATA
(ESC)= STOP
CTRL F1 = BACKUP
CTRL F2= NEXT
CTRL F3= AUTOMATIC
CTRL F6= FORWARD

FIG. 13
ANALYSIS SCREEN

PATIENT IDENTIFICATION
ACCESSION NUMBER
DNA INDEX BASED ON 7.18 PICOGRAMS

DISTRIBUTION OF DNA MASS

CELL COUNT (y-axis: 20, 16, 12, 8, 4, 0)

CELL MASS (PICOGRAM) (x-axis: 0, 8, 16, 24, 32)

AREA 1   C
AREA 2   C

OPTION LIST

LIGHT LEVEL   0
CELL TYPES N 1234L

MAIN PEAK
    MASS   14.6   pg.
    INDEX   2.03
    AREA   100.0 pm2

SECOND PEAK
    MASS   .0   pg.
    INDEX   .0
    AREA   .0   pm2

COUNT
    TOTAL   24
    SHOWN   24
    OFF SCALE   0
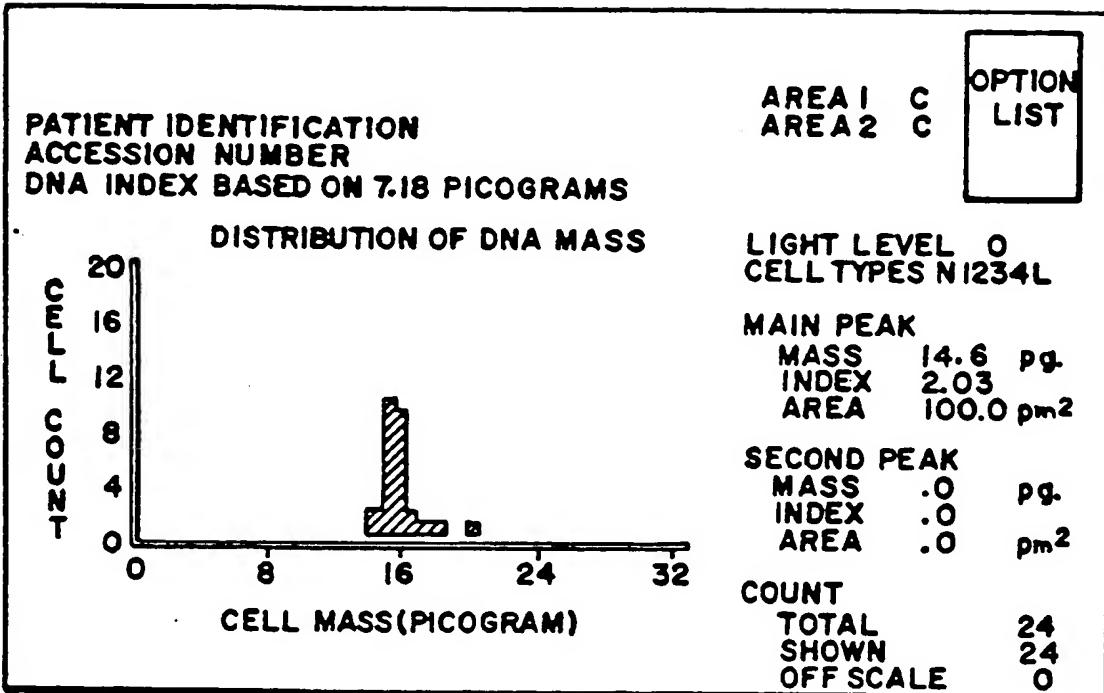
FIG. 14
X, Y FIELD COORDINATES SCREEN

FIELD#	X	Y	FIELD#	X	Y	FIELD#	X	Y	
1	1243	0218							OPTION LIST
2	1236	0210							
3	1235	0039							
4	1183	0078							

FIG. 15

A10 — MAIN MENU

A12 — LABEL

CALIBRATE — A14

ANALYZE — A16

A18 — HELP

EXIT — A20

FIG. 16

A22 — CALIBRATION MENU

A24 — SET-LIGHT

SET — A26

FOCUS — A32

MEASURE — A36

X Y — A38

ANALYZE — A28

CLEAR — A30

HELP — A34

MAIN — A40

FIG. 17

A42 — ANALYSIS MENU

A44 — CLASSIFY

FOCUS — A46

CK-LIGHT — A48

SELECT 2ND — A50

AREA 1-2 — A52

DISPLAY-XY — A56

CLEAR-XY — A60

REPORT — A64

SCALE — A54

BOUNDRY — A58

HELP — A62

MAIN — A66

FIG. 18

CALIBRATE MEASURE — A100

0	9	CTRL/F1	CTRL/F2
ACCEPT CALIBRATION OBJECT A102	REJECT CALIBRATION OBJECT A108	MOVE IDENT TO PREVIOUSLY MEASURED OBJECT A112	MOVE IDENT TO NEXT UNMEASURED OBJECT A116

ENTER	CTRL/F3	CTRL/F6	ESC
TERMINATE AND SAVE DATA A104	MEASURE AUTOMATICALLY A106	MOVE IDENT TO NEXT OBJECT A110	TERMINATE AND DO NOT SAVE DATA A114

FIG. 19

ANALYSIS CLASSIFY — A200

0-5	9	CTRL/F1	CTRL/F2
CLASSIFY CURRENT OBJECT A202	REJECT CURRENT OBJECT A204	MOVE IDENT TO PREVIOUSLY MEASURED OBJECT A206	MOVE IDENT TO NEXT UNMEASURED OBJECT A208

CTRL/F3	CTRL/F4	CTRL/F5	CTRL/F6
AUTOMATIC CLASSIFICATION A210	CELL CUTTING FUNCTION A214	SELECT OBJECT A218	MOVE IDENT TO NEXT OBJECT A220

ENTER	ESC
A212 TERMINATE AND SAVE DATA	A216 TERMINATE AND DO NOT SAVE DATA

FIG. 20

FIG. 21

FIG. 22

DISTRIBUTION OF LIGHT INTENSITY

GRAY LEVEL

FIG. 23

FIG. 24

FIELD ARRAY

CELL OBJECT DATA
A
CELL OBJECT DATA
B
CELL OBJECT DATA
C

FIG. 24A

FIELD ARRAY

FIELD ARRAY
A
FIELD ARRAY
B
FIELD ARRAY
C
FIELD ARRAY
D

FIG. 24B

CELL OBJECT

ENTRY POINT
NUMBER OF PIX
X,Y MIN-MAX
PERIMETER PIX COUNT
SUM OF PIX O D
CLASS
X,Y FIELD COORDINATES

FIG. 24C